

# Whatman®



Components for Diagnostic Kit Developers  
Solutions for Everyday Problems

# INTRODUCTION

## **COMPONENTS FOR DIAGNOSTIC KIT DEVELOPERS SOLUTIONS FOR EVERYDAY PROBLEMS**

For today's diagnostic kit manufacturers, time to market is more critical than ever. You seek cost-efficient business partnerships that yield consistent product performance and quality with increasingly aggressive rates of development. In essence, you require experienced single-source vendors with extensive product lines that address the full spectrum of applications.

And that's why so many diagnostic companies partner with Whatman.

We offer a comprehensive family of high-quality products, from components for lateral-flow test devices to sample collection and transport materials to sample preparation filter media and devices. For each integral step of a test, Whatman has the optimal product to fit your needs.

By combining the highest-quality standardized solutions with unsurpassed technical expertise and support, we enable you to optimize your product offerings quickly and cost effectively. We thereby accelerate your time to market and maximize your competitive advantage.



# ORDERING INFORMATION

<b>FUSION 5™</b>		
<b>Product</b>	<b>Dimensions</b>	<b>Cat. No.</b>
FUSION 5	6 cm x 50 m	8151-6755
	210 mm x 297 mm	8151-6621*
*50 sheets per pack.		
<b>Lateral Flow Nitrocellulose Membranes</b>		
<b>Product</b>	<b>Dimensions</b>	<b>Cat. No.</b>
<b>Immunopore®</b>		
Immunopore SP	25 mm x 50 m	78 316 403
Immunopore FP	25 mm x 50 m	78 336 403
Immunopore RP	25 mm x 50 m	78 356 403
<b>PRIMA™</b>		
PRIMA 125	25 mm x 300 mm	10 549 730*
	25 mm x 50 m	10 549 729
PRIMA 85	25 mm x 300 mm	10 549 726*
	25 mm x 50 m	10 549 725
PRIMA 60	25 mm x 300 mm	10 549 724*
	25 mm x 50 m	10 548 312
PRIMA 40	25 mm x 300 mm	10 549 712*
	25 mm x 50 m	10 549 713
<b>AE</b>		
AE 100	25 mm x 300 mm	10 549 687*
	25 mm x 50 m	10 547 017
AE 99	25 mm x 300 mm	10 549 880*
	25 mm x 50 m	10 548 081
AE 98 Fast	25 mm x 300 mm	10 549 688*
	25 mm x 50 m	10 547 097
AE 98	25 mm x 300 mm	10 549 689*
	25 mm x 50 m	10 548 109
*10 strips per pack.		
<b>Blood Separation</b>		
<b>Product</b>	<b>Dimensions</b>	<b>Cat. No.</b>
LF1	12 mm x 50 m	8121-1250
	17 mm x 50 m	8121-1750
	22 mm x 50 m	8121-2250
MF1	12 mm x 50 m	8122-1250
	17 mm x 50 m	8122-1750
	22 mm x 50 m	8122-2250
	27 mm x 50 m	8122-2750
VF1	17 mm x 50 m	8123-1750
VF2	12 mm x 50 m	8124-1250
	17 mm x 50 m	8124-1750
<b>Conjugate Release</b>		
<b>Product</b>	<b>Dimensions</b>	<b>Cat. No.</b>
Rapid 24	12 mm x 50 m	8131-1250
	17 mm x 50 m	8131-1750
	22 mm x 50 m	8131-2250
	27 mm x 50 m	8131-2750
Rapid 27	12 mm x 50 m	8132-1250
	17 mm x 50 m	8132-1750
	22 mm x 50 m	8132-2250
	27 mm x 50 m	8132-2750
Standard 14	12 mm x 50 m	8133-1250
	17 mm x 50 m	8133-1750
	22 mm x 50 m	8133-2250
	27 mm x 50 m	8133-2750
Standard 17	12 mm x 50 m	8134-1250
	17 mm x 50 m	8134-1750
	22 mm x 50 m	8134-2250
	27 mm x 50 m	8134-2750

Sample Wick and Absorbent		
Product	Dimensions	Cat. No.
CF1	12 mm x 50 m	8111-1250
	22 mm x 50 m	8111-2250
CF3	12 mm x 50 m	8113-1250
	17 mm x 50 m	8113-1750
	22 mm x 50 m	8113-2250
	27 mm x 50 m	8113-2750
CF4	12 mm x 50 m	8114-1250
	17 mm x 50 m	8114-1750
	22 mm x 50 m	8114-2250
CF5	17 mm x 50 m	8115-1750
	22 mm x 50 m	8115-2250
	27 mm x 50 m	8115-2750
CF6	22 mm x 50 m	8116-2250
	27 mm x 50 m	8116-2750
CF7	12 mm x 50 m	8117-1250
	17 mm x 50 m	8117-1750
	22 mm x 50 m	8117-2250
	27 mm x 50 m	8117-2750
Standard 14	12 mm x 50 m	8133-1250
	17 mm x 50 m	8133-1750
	22 mm x 50 m	8133-2250
	27 mm x 50 m	8133-2750
Standard 17	12 mm x 50 m	8134-1250
	17 mm x 50 m	8134-1750
	22 mm x 50 m	8134-2250
	27 mm x 50 m	8134-2750
Rapid 24	12 mm x 50 m	8131-1250
	17 mm x 50 m	8131-1750
	22 mm x 50 m	8131-2250
	27 mm x 50 m	8131-2750
Rapid 27	12 mm x 50 m	8132-1250
	17 mm x 50 m	8132-1750
	22 mm x 50 m	8132-2250
	27 mm x 50 m	8132-2750
Flow-through Nitrocellulose Membranes		
Product	Dimensions	Cat. No.
BA 79	15 cm x 15 cm	10 402 094*
BA 83	15 cm x 15 cm	10 549 686*
BA 85	15 cm x 15 cm	10 549 685*
FT 020	15 cm x 15 cm	10 549 684*
FT 045	15 cm x 15 cm	10 549 683*
FT 060	15 cm x 15 cm	10 549 682*
*10 sheets per pack.		
Sample Collection		
Product	Dimensions	Cat. No.
CF3	12 mm x 50 m	8113-1250
	17 mm x 50 m	8113-1750
	22 mm x 50 m	8113-2250
	27 mm x 50 m	8113-2750
CF5	17 mm x 50 m	8115-1750
	22 mm x 50 m	8115-2250
	27 mm x 50 m	8115-2750
CF7	12 mm x 50 m	8117-1250
	17 mm x 50 m	8117-1750
	22 mm x 50 m	8117-2250
	27 mm x 50 m	8117-2750

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# INTRODUCTION TO DETECTION

## DETECTION, THE KEY IN DIAGNOSTIC SCIENCE

Assays to look for ever-lower levels of antigen are under development. Target sensitivity for tests today is lower than it was yesterday, and the tests of tomorrow will be even lower. These constantly decreasing sensitivity levels necessitate greater and greater demands upon the detection technology, platform and materials. Whatman is striving to meet tomorrow's needs with the materials available today.

A number of platforms and formats are used, and each has a new set of requirements. In rapid assays, both lateral-flow and flow-through assay developers are looking for tests that are ultrasensitive or quantitative. The membranes and fibrous components must have greater consistency, higher protein binding and lower background and must have better handling characteristics than ever before. The increasing use of new labels, such as quantum dots, fluorescent dyes and magnetic particles, is introducing new variables that have never previously been addressed. Protein arrays are revolutionizing multiplexed assays. Never before has it been possible to get so much data of clinical utility in a simple and rapid way. Increasing use of dipstick or colorimetric assays, with the presence of the analyte causing a change in color on a pad, is providing a new and simple way to test for a range of small analytes in clinical and environmental applications.

In every format whose result is read directly off the substrate, the needs for greater performance are placing increasing demands upon the substrates. The characteristics that were suitable for the tests of yesterday will not be adequate for the assays of tomorrow. Whatman is always looking to introduce new products that answer the needs of diagnostic test manufacturers. That's why we offer new conjugate pads that are more stable; blood separators that are more consistent and require lower sample volumes; membranes with lower variation; absorbents that limit backflow; protein arrays with simple antigen binding; colorimetric assay substrates that do not cause interference; and FUSION 5™, a new platform for rapid assays. The central concern in the design of Whatman materials is the assay developer's needs. Therefore, Whatman materials are the best choice to help you solve the problems of today to meet the demands of the future.



# INTRODUCTION TO LATERAL FLOW

## WHATMAN LATERAL-FLOW TECHNOLOGY:

### Many Products, Multiple Benefits

With a vast array of products and innovative designs, Whatman is the leader in lateral-flow technology. From our ground-breaking FUSION 5™, which performs five functions of a lateral-flow test, to our wide range of novel conjugate release products that improve performance without an

additional blocking step, diagnostic manufacturers rely on Whatman diagnostic products.

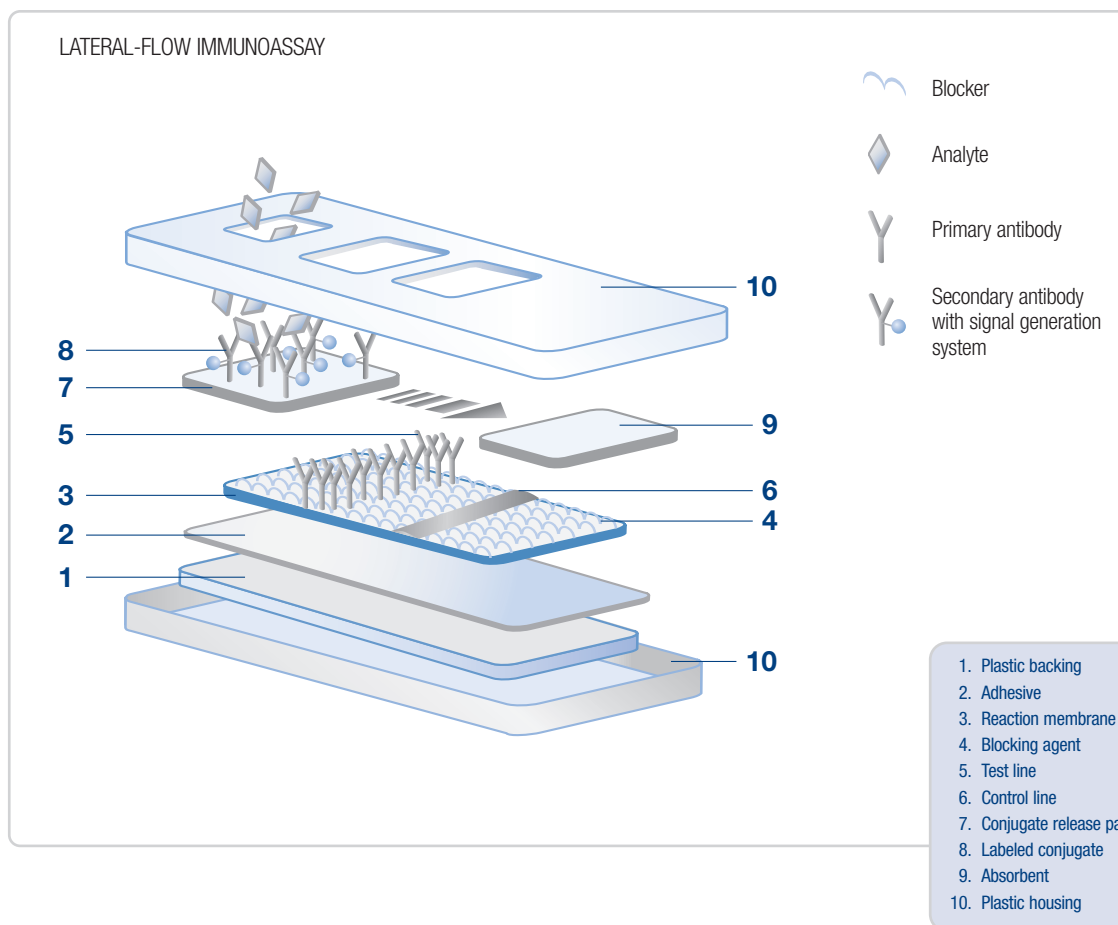
Our quality product line ensures accurate and reproducible results. From our glass fiber materials to our nitrocellulose membranes, Whatman products are easy to use.

### Lateral-Flow Immunoassays: A Primer

Developments in lateral-flow immunoassay systems allow for single-step assays that require only the addition of a sample. This sample flows through the device and comes in contact with dried reagents,

usually a tagged secondary antibody. The antibody and analyte migrate to a capture zone of membrane-immobilized antibody. Any unreacted tagged antibody flows past the capture zone to an end-of-assay indicator.

FUSION 5 provides a new platform for lateral-flow assays. In contrast to the traditional techniques that use multiple materials for the strip production, FUSION 5 allows the entire test strip to be built on a single material, simplifying test design and reducing manufacturing costs by up to 60%.



# FUSION 5™

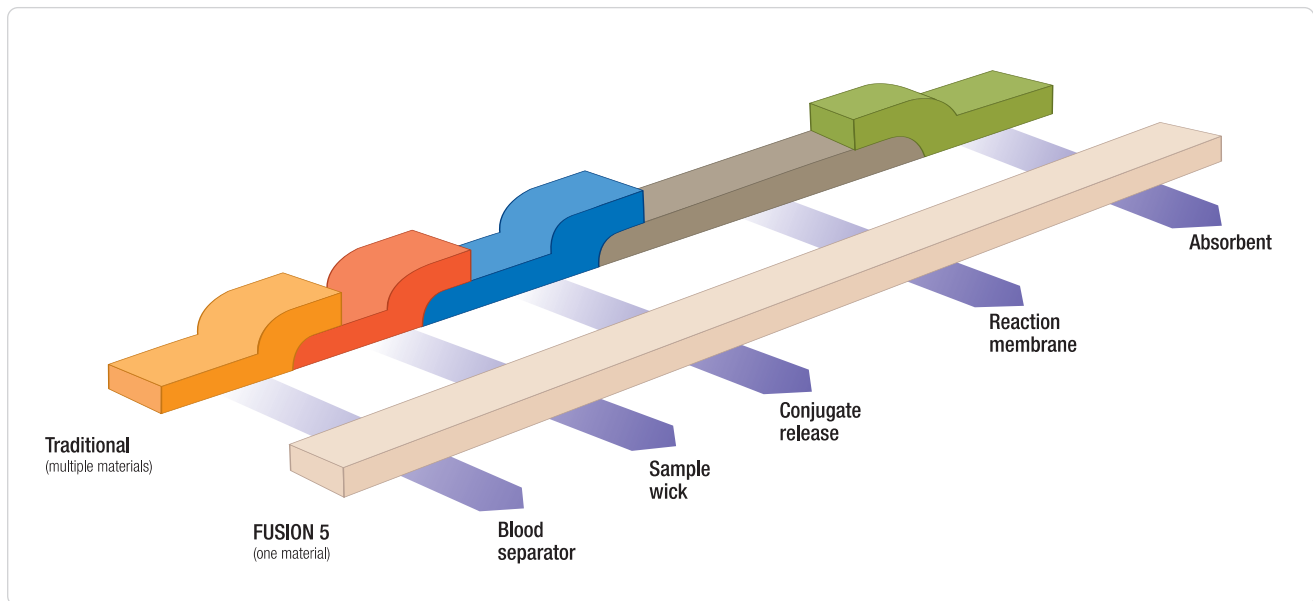
## ONE MATERIAL, FIVE FUNCTIONS

Developers of rapid tests have always faced the problem of selecting lateral-flow component materials and getting them to perform effectively together in a test. Glass fiber, polyester, nitrocellulose and cellulose—where do you start and which materials do you choose? Now we have a solution.

Features	Benefits
Test development is faster, easier	Saves time and money
Material is hydrophilic	No blocking required
Only one material to optimize	Able to develop best production methods
Eliminates component contact issues	Ensures accurate test results
Test is highly sensitive	Low background eliminates false-positive results
Greater reliability with a single material	Consistent performance
Fast flowing, with wicking rates under 40 seconds for a 4-cm strip	Quick, accurate results
Manufacturing is easier, faster, more efficient	No assembly required; saves up to 60% of manufacturing costs
Quality control of only one material	No lot-to-lot variability
Simplifies purchasing	Saves time and money

Based on our proprietary FUSION (single-layer matrix) technology, FUSION 5 is a single material that performs all of the functions of a lateral-flow strip. FUSION 5

can be used for a wide range of tests, simplifying manufacturing and reducing costs.



FUSION 5 VS. TRADITIONAL LATERAL FLOW FORMAT

### Typical Test Data

Thickness (µm @ 53 kPa)	Klemm Wicking (s/4 cm)	Max Pore Size (µm)	Water Absorption (mg/cm²)	Particle Retention (µm)	% Release of Gold Conjugate	% Release of Latex Conjugate	% of Available Serum Obtained
370	38	11.0	40	2.3	> 94	> 83	86

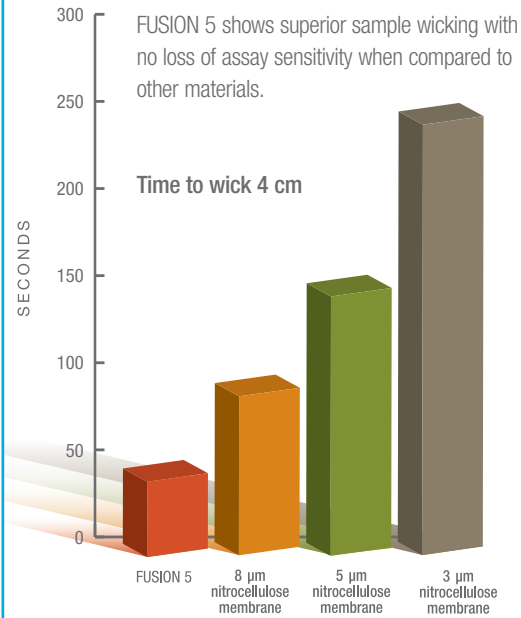
## 1. BLOOD SEPARATOR

% Available to Test*	
Volume Serum	86
Total Protein	98
IgG	99
Cholesterol	101

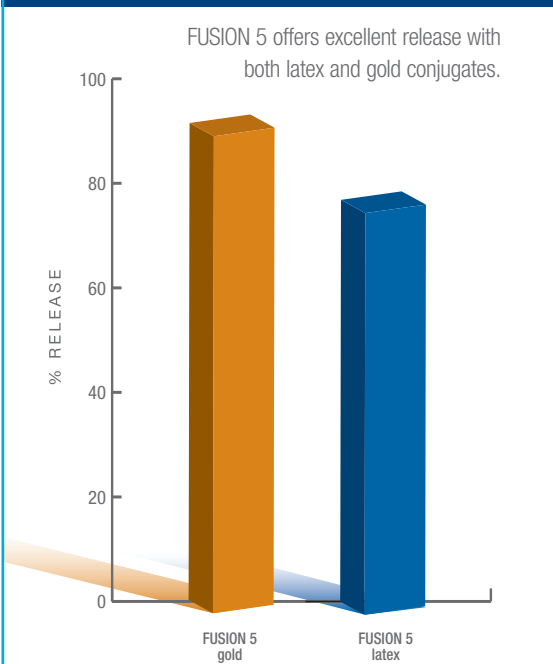
\*Versus spun serum

FUSION 5™ offers highly efficient blood separation with no analyte interference.

## 2. SAMPLE WICK

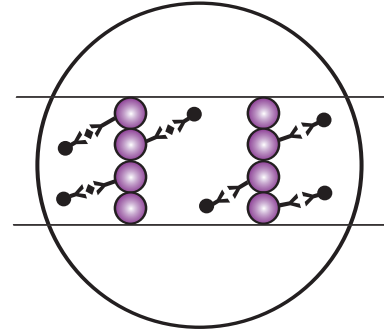


## 3. CONJUGATE RELEASE



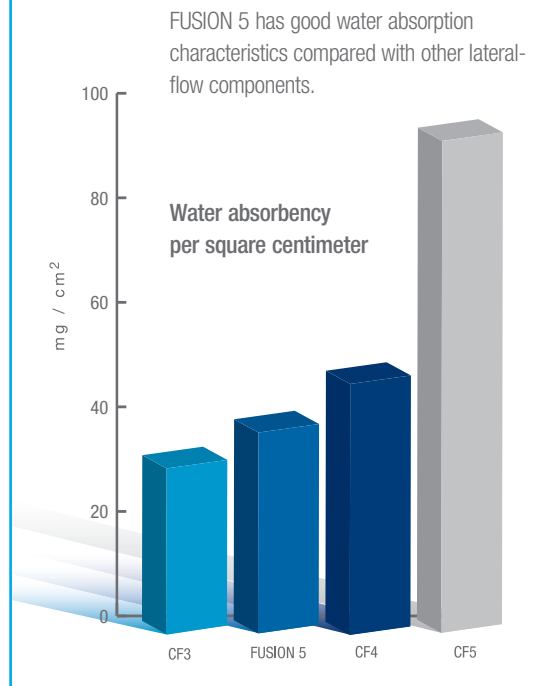
## 4. REACTION MEMBRANE

When beads of approximately 2 µm are applied to FUSION 5, they bind. Capture reagents bound to the immobilized latex beads combine with conjugate and analyte to give intense capture lines.



An alternative approach can be used. FUSION 5 has a negative charge. Applying protein at a low pH (when the protein has a positive charge) will result in immobilization of the applied protein after drying.

## 5. ABSORBENT



# NITROCELLULOSE MEMBRANES

## IMMUNOPORE® AND PRIMA™

Nitrocellulose membranes are a key functional part of lateral-flow assays. The membrane must provide sufficient protein binding to enable the production of a sharp and intense capture line, but at the same time the level of nonspecific background must be low for easy interpretation of results.

Nitrocellulose membranes are available in a range of wicking rates and formulations. It has long been known that the wicking rate of a membrane has a significant impact upon test sensitivity. However, it has also been shown that the membrane formulation plays a very large part in the way the membrane functions. For this reason Whatman is pleased to offer a range of membranes with different wicking rates and formulations to address both sensitivity and speed.

### For Sensitivity: Immunopore

Immunopore is the membrane of choice for producing the most sensitive assays. This membrane, available in three wicking rates directly cast onto a plastic backing,

offers unparalleled sensitivity and consistency. For anyone working with critical assays, such as for infectious disease, whose reproducibility, stability and accuracy are paramount, the Immunopore range provides the best solution.



## Product Range

Product Name	Mylar Cast	Uses
Immunopore RP	Yes	A fast-wicking membrane with excellent sensitivity for use with all sample types. The ideal general-purpose membrane.
Immunopore FP	Yes	A membrane with moderate wicking rate and excellent sensitivity for intermediate-viscosity sample types.
Immunopore SP	Yes	A membrane that gives the strongest and sharpest capture lines for use with low-viscosity samples.

Features	Benefits
Proprietary polymer ensures rapid rewetting and low background signal	Eliminates protein-binding interference
Very sensitive, with high intensity at capture line	Low positives easy to read
High consistency	Test development predictable with outstanding between-batch consistency for lateral wicking
Long shelf life	No significant performance loss within 18 months of storage
Versatile	Appropriate for all tests with precise results regardless of capture reagent or analyte type

## Typical Test Data

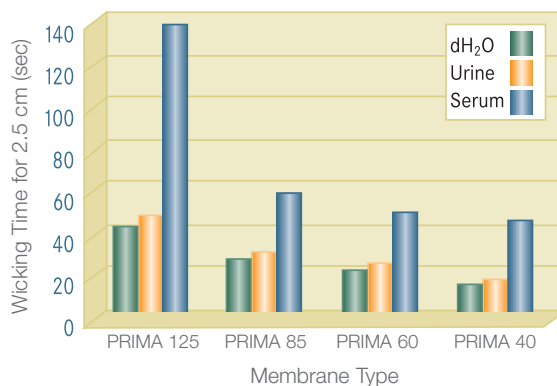
Description	Capillary Rise* (s/4 cm)	Caliper† (µm)
Immunopore RP	85–115	200
Immunopore FP	110–150	200
Immunopore SP	160–220	200

\*Cross web measured with water (rise time with serum or other liquids will differ).  
 † Including standard polyester backing, which is 100 µm thick.

### For Speed: PRIMA™

PRIMA membranes are also available as a plastic cast material. PRIMA membranes offer much faster wicking rates, giving faster assays. However, their ultimate sensitivity is less than that of Immunosorb®. PRIMA provides an easy answer for developers who want the fastest result or who are working with samples that are highly viscous or that contain high particulate loading.

### Capillary Rise/Wicking Time of PRIMA Direct-Cast Membranes as Related to Sample Type



Strips 25 x 10 mm were cut from each membrane in the cross-web direction and placed in 100 µL of dH<sub>2</sub>O, urine or serum. Time was measured in seconds from the moment the strip touched the sample until the moving front reached the end of the strip.

### Product Range

Product Name	Mylar Cast	Uses
PRIMA 40	Yes	The fastest-wicking membrane for use with the most viscous samples or samples that contain high levels of particulates
PRIMA 60	Yes	A very fast wicking membrane for use with highly viscous samples, e.g., undiluted serum
PRIMA 85	Yes	A general-purpose membrane for use with most sample types
PRIMA 125	Yes	A membrane with very easy handling requirements that gives good line intensity for use with low-viscosity samples

Features	Benefits
Direct-cast, large-pore nitrocellulose membrane with exceptional cross-web and down-web consistency	Superior sensitivity, ideal for highly specific applications
No treatments post manufacture	Uniquely homogenous membrane
Rapid rewetting	Easy absorption of all sample types
Extensive range of flow rates	Meets the requirements of even the most difficult assays

#### Typical Test Data

Description	Capillary Rise* (s/4 cm)	Caliper† (µm)
PRIMA 40	35–55	200
PRIMA 60	50–75	200
PRIMA 85	70–105	200
PRIMA 125	100–150	200

\*Cross web measured with water (rise times with serum or other liquids will differ).

†Including standard polyester backing 100 µm thick.

# NITROCELLULOSE MEMBRANES

## AE MEMBRANES

Constructed of 100% nitrocellulose with no treatments post manufacture, the AE family of membranes offers a level of purity and performance that cannot be seen in post-treated materials. The AE membranes have been extensively used in the lateral-flow industry for

the past 20 years and have become the standard for manufacturers on every continent.

The AE membranes, along with the plastic-backed FF membranes, have been used since the development of the original lateral-flow tests. There is an unrivaled history of successes and experience for the optimization of these products in assays.

### For Purity: AE and FF

AE membranes are unsupported, which means either the belt or air side of the membrane can be used. They can be used in either lateral-flow or flow-through assays.

FF membranes are a part of the AE family that are directly cast onto a plastic film. The plastic cast membranes are ideal for lateral-flow assays that require faster wicking rates.

## Product Range

Product Name	Mylar Cast	Uses
AE 100	No	A very fast wicking membrane for use with highly viscous samples, e.g., undiluted serum
AE 99	No	A general-purpose membrane for use with most sample types giving a good combination of sensitivity with fast wicking
AE 98 Fast	No	A sensitive membrane that has a fast wicking rate for use with most sample types
AE 98	No	An unsupported membrane that gives good line intensity for use with low-viscosity samples
FF60	Yes	A very fast wicking membrane for use with highly viscous samples, e.g., undiluted serum
FF85	Yes	A general-purpose membrane for use with most sample types
FF125	Yes	A plastic cast membrane that gives the strongest and sharpest capture lines for use with low-viscosity samples

Features	Benefits
Constructed of 100% nitrocellulose with no treatments post manufacture	Complete lateral flow system optimization and consistent performance
Excellent membrane purity	Full control of test system additive levels
Low background	Clearer test results
Extensive range of flow rates	Meets the requirements of even the most difficult assays

### Typical Test Data

Description	Capillary Rise* (s/4 cm)	Caliper† (µm)
AE 100	90–120	120
AE 99	120–160	120
AE 98 Fast	110–160	120
AE 98	160–210	120
FF60	45–75	200
FF85	65–105	200
FF125	100–150	200

\*Cross web measured with water (rise time with serum or other liquids will differ).  
 † Including standard polyester backing which is 100 µm thick.

# BLOOD SEPARATION

## RAPID SEPARATION OF WHOLE BLOOD

Given the increasing demand for whole-blood assays, Whatman offers a family of blood separators specifically designed to meet the strict requirements of the rapid diagnostic market. A full range of separator grades allows for faster and easier product development.

### Product Range

Since product selection for specific blood separation applications can be complex, please consult the “Where to Start” chart below for typical applications.

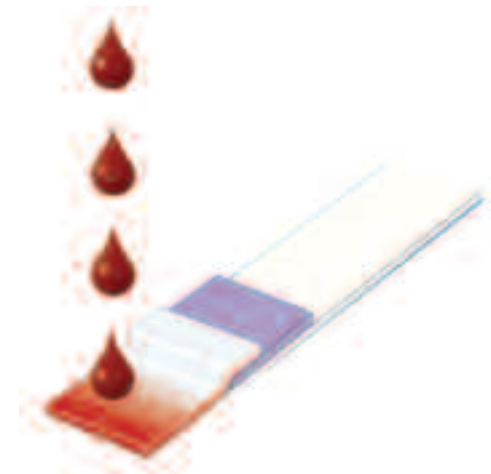
**LF1** – Designed for use in lateral-flow assays. Works well with one drop of whole blood.

**MF1** – Used for lateral or vertical-flow assays. Typically used for whole-blood volumes around 100 µL.

**VF1** – Used for vertical-flow assays. Normally used in a multilayer format for separating larger volumes of blood.

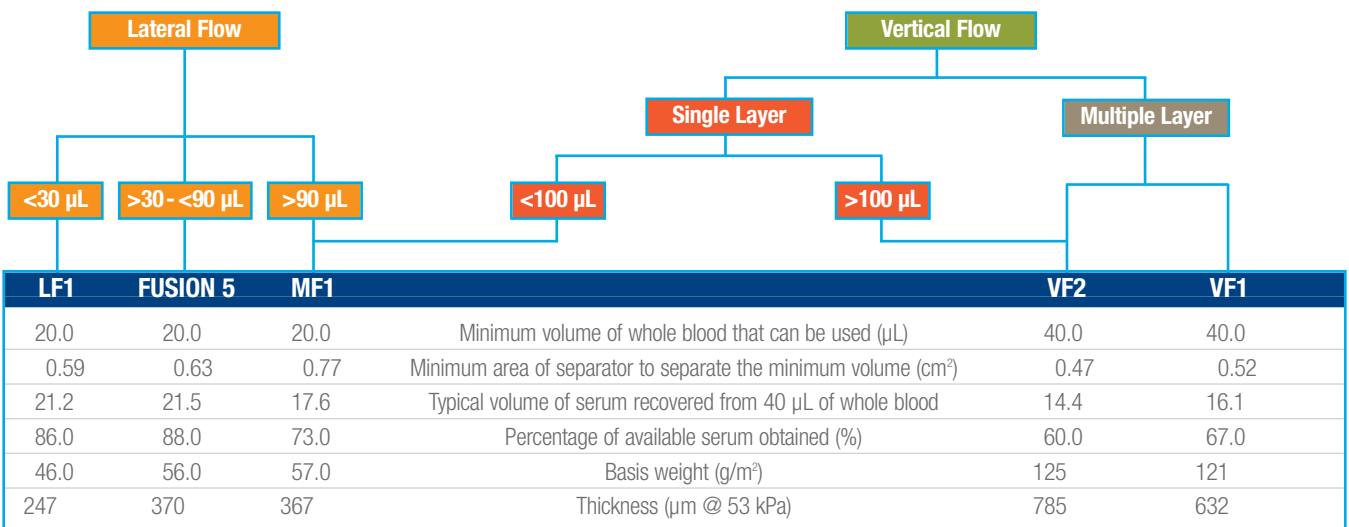
**VF2** – Vertical separator used as single or multiple layers for separation of a wide range of blood volumes.

**FUSION 5™** – Can be used as a lateral-flow blood separator with two drops of whole blood.



Features	Benefits
Separation in 30–120 seconds	Rapid assays save time
No appreciable red cell hemolysis	Improved reproducibility
Consistency of materials	Reliability
Materials suitable for use in a range of tests	Flexibility in test design
Choice of separation times	Allows for test optimization
Separators appropriate for range of blood volumes	Separator is fully optimized to give the fastest blood volumes separation rate for the volume of blood available

## Where to Start



# CONJUGATE RELEASE

## RAPID RELEASE

Conjugate release pads are critical to lateral-flow assays. To ensure consistent performance and a strong test line, the conjugate must dry without damage or aggregation and release rapidly when the sample is applied.

For consistent, reliable results, diagnostic manufacturers rely on Whatman products.

Whatman conjugate release pads do not require treatment prior to conjugate application, as they are inherently hydrophobic. The open structure of the material allows rapid penetration by both conjugate and sample.



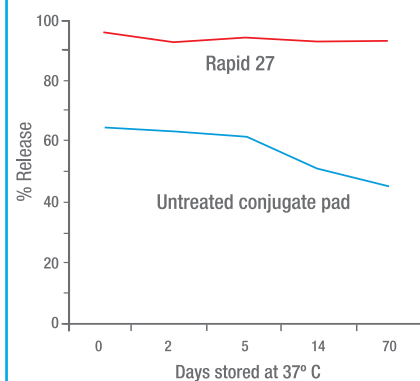
## Rapid 24/Rapid 27 Treated Bound Glass Fiber Pad

No additional blocking step is required, which accelerates assay development and simplifies manufacturing. These products typically release more conjugate than untreated pads, reducing reagent costs. Pads rewet rapidly, and capture line intensity is increased, significantly improving performance. Rapid 24 has higher absorption capacity and greater flexibility, while Rapid 27 has greater tensile strength.

## Standard 14/Standard 17

These untreated grades are ideal for conjugate pad optimization for particularly sensitive assays. They are manufactured to enable optimization, giving the same results every time. Pads rewet quickly and naturally without surfactant treatment, facilitating simplified production. Standard 14 has higher absorption capacity and greater flexibility, while Standard 17 has greater tensile strength for easier manufacturing.

Release of gold colloid from conjugate pads after storage at 37° C

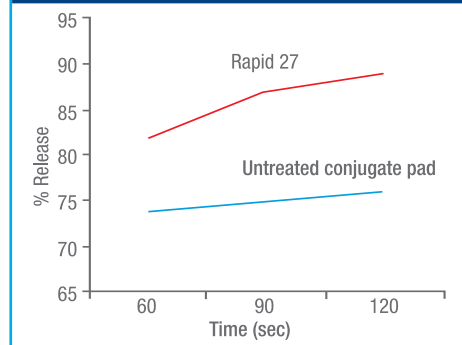


Features	Benefits
Higher levels of conjugate release	Less waste means reduced reagent costs
Higher capture line intensity, as more conjugate gets to the capture line	Improved sensitivity
Pad rewets naturally and rapidly every time	Improved consistency
Every batch tested for performance	Assured quality control and optimum performance

## Typical Test Data

Grade	Thickness (µm @ 53 kPA)	Absorption Capacity (mg/cm <sup>2</sup> )	Max. Pore Size (µm)	% Release of Gold Conjugate (after 90 sec)
Rapid 24	340	55	22	89
Rapid 27	365	40	22	86
Standard 14	355	55	23	75
Standard 17	370	35	23	75

Release of gold colloid from conjugate pads



# SAMPLE WICK AND ABSORBENT

## THE BEGINNING AND END OF ASSAYS

Sample wicks begin the assay by transporting samples from the point of application to the test components. Absorbent sinks at the downstream end of tests control sample flow along the strip.

To ensure that your assays begin without complications, Whatman offers a complete range of high-quality sample wick materials. Whatman has also developed sinks with excellent wicking characteristics that give rise to greater consistencies.

### Grade CF6 – A Solution to Conjugate Backflow

Backflow has been a problem for manufacturers of lateral-flow tests for many years. In many assays, as the test

stands after being run, the sample and any conjugate it contains have a tendency to run back down the membrane from the absorbent pad. This can give rise to false-positive results. If CF6 is used as the conjugate pad, there is less chance of the conjugate running back down the strip. This unique performance is a function of the proprietary manufacturing process. Only CF6 can provide a simple answer to the problem of conjugate backflow.

## Product Range

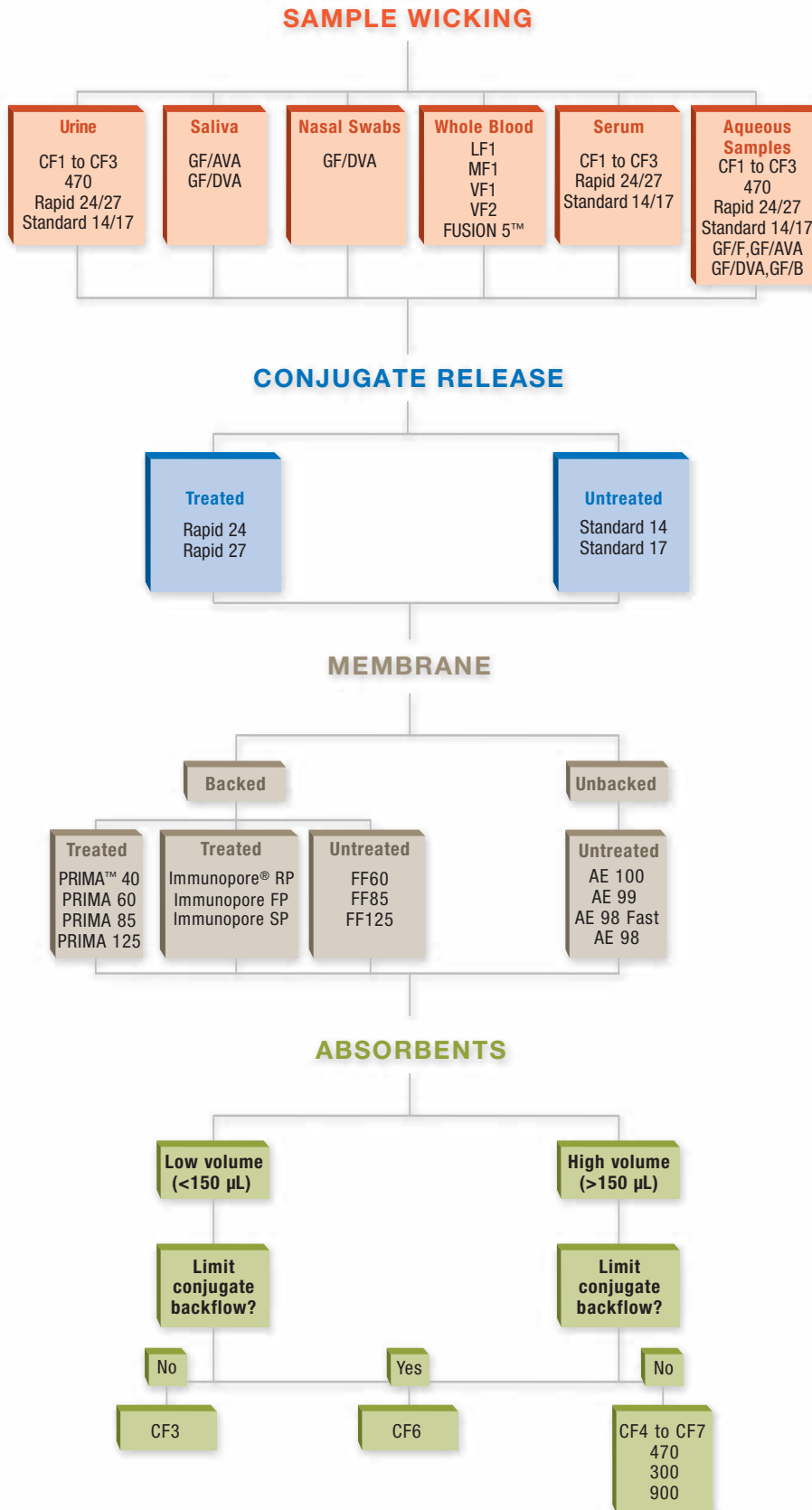
Sample Wick and Absorbent				
Product	Material	Properties	Sample Wick	Absorbent
CF1	100% cotton linter	Light, thin grade suitable for small volume	●	
CF3, CF4 & 470	100% cotton linter	Medium weight	●	●
CF5 & CF10	100% cotton linter	Higher absorbency for medium sample volumes and flow rates		●
CF6	Mixture of glass fiber and cotton	Higher absorbency and faster flow rate than CF5. <b>Solves conjugate backflow.</b>		●
CF7, 900 & 300	100% cotton linter	Thick materials suitable for high sample volume		●
Standard 14 & 17	Bound glass fiber	Glass fiber material for faster flow than cotton, with lower sample retention	●	
Rapid 24 & 27	Treated bound glass fiber	Treated glass fiber material for good rewetting of membranes and improved conjugate release without interfering with assay sensitivity	●	
GF/AVA	Bound glass fiber	Bound glass fiber to remove small particles from sample before entering the strip, reducing membrane blocking	●	
GF/DVA	Bound glass fiber	Bound glass fiber, faster flowing than GF/AVA, particularly suitable for saliva samples	●	
LF1, MF1 & VF2	Bound glass fiber	Suitable for use with whole blood or serum samples	●	
VF1	Unbound glass fiber	Suitable for use with whole blood or serum samples	●	
Features		Benefits		
Consistent absorbency and wicking rates		Superior test-to-test reproducibility		
Products manufactured in controlled environments from highest-quality materials		No false results due to sample contamination		
Low protein binding		Minimal loss of analyte, so test sensitivity is maintained		
Naturally hydrophilic		Rapid rewetting after prolonged storage		
Wide range of thickness, absorbency and wicking rate		Compatible with most styles of housings		
Minimal leakage along the strip		No contamination of test results		

## Typical Test Data

Grade	Thickness (µm @ 53 kPA)	Wicking Rate (s/4 cm)	Water Absorption (mg/cm <sup>2</sup> )
CF1	176	187	16
CF3	322	161	31
CF4	482	65	46
CF10	490	92	42
470	840	77	78
CF5	954	75	98
CF6	1370	65	128
CF7	1873	35	198
900	1830	34	204
300	2590	32	240

Grade	Thickness (µm @ 53 kPA)	Wicking Rate (s/4 cm)	Water Absorption (mg/cm <sup>2</sup> )
Standard 14	355	42	55
Standard 17	370	47	35
Rapid 24	340	38	55
Rapid 27	365	39	40
GF/AVA	299	65	32
GF/DVA	758	44	98
LF1	247	43	20
MF1	367	58	42
VF1	632	41	102
VF2	785	55	98

# LATERAL FLOW SELECTION CHART



## WHATMAN RECOMMENDS

Rapid 27

Rapid 27

Immunopore RP

CF6

# FLOW-THROUGH ASSAYS

## HIGH BINDING CAPACITY; EXCEPTIONAL PERFORMANCE

In a flow-through assay the sample is applied directly to the membrane surface and is allowed to wick through the membrane into an absorbent paper below. The membrane of choice for flow-through tests is a paper cast nitrocellulose, such as the

FT range from Whatman. In paper cast materials, the membrane is cast directly onto the surface of a high-quality cellulose paper. This simplifies manufacturing and reduces the chance of test failure due to poor contact between the components. Small-pore unsupported membranes, such as BA83 and BA85, can be used; however, they have

to be carefully encapsulated to ensure good contact between the membrane and the absorbent to give good flow.

### Absorbents

The absorbents used for flow-through assays must wick fast and be highly water absorbent. The volumes of liquids used in flow-through assays can be much higher than those in lateral flow. Thicker cellulose materials with fast wicking are therefore the material of choice.

## Product Range

Product	Properties
BA	Highly sensitive small-pore membrane with large surface area and high protein-binding capacity
FT	Flow-through membranes direct-cast on absorbent backing paper; bilayer media are frequently used in flow-through assay platforms

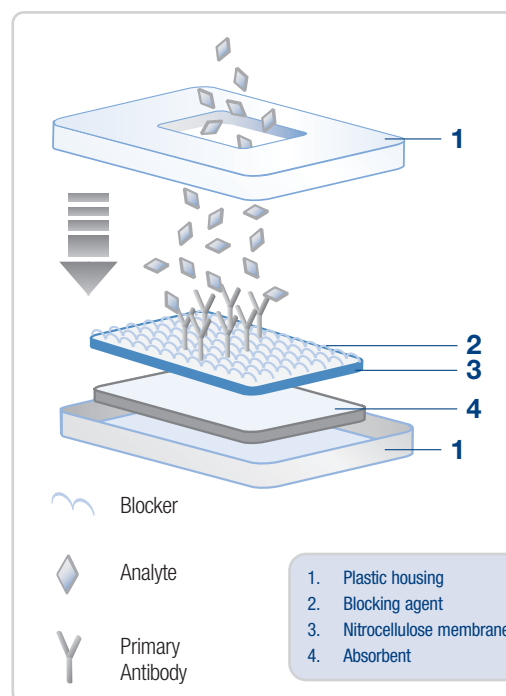
  

Features	Benefits
Manufactured for vertical-flow assays	Eliminates problems caused by capillary rise
Small pore structure	Accurate results; low nonspecific binding; greater sensitivity
Increased surface area	Higher binding capacity and sensitivity with superior signal-to-noise ratios
100% pure nitrocellulose	Guarantees the highest possible binding capacity
Specified to your application	Customized to meet your needs

## Typical Test Data

Grade	Thickness ( $\mu\text{m}$ @ 53 kPa)	Wicking Rate (s/4 cm)	Water Absorption (mg/cm <sup>2</sup> )
CF4	482	65	46
CF5	954	75	98
CF6	1370	65	128
CF7	1873	35	198
900	1830	34	204
300	2590	32	240

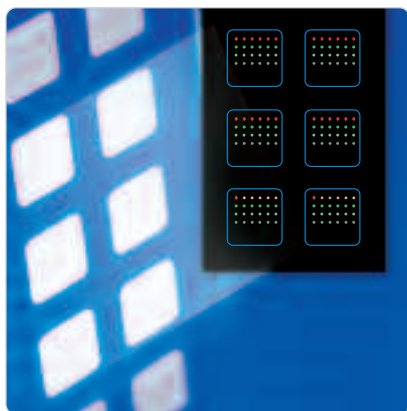
Grade	Pore Size ( $\mu\text{m}$ )	Caliper ( $\mu\text{m}$ )	Protein Binding ( $\mu\text{m IgG/cm}^2$ )
Unsupported membranes			
BA 79	0.10	120	140
BA 83	0.20	120	125
BA 85	0.45	120	110
Paper-backed membrane			
FT 020	0.20	500–600	125*
FT 045	0.45	500–600	110*
FT 060	0.60	500–650	90*
*Protein binding of nitrocellulose layer			



# INTRODUCTION TO PROTEIN ARRAYS

## THE WORLD'S LEADING SURFACE FOR PROTEIN MICROARRAY APPLICATIONS

Whatman FAST® Slides are the world's leading surface for protein microarray applications. Our proprietary 3-D nitrocellulose-coated slides create a highly protein-binding surface.



### FAST SLIDE FEATURES:

- Superior protein-binding capacity and reproducibility
- Highest sensitivity and dynamic range
- Excellent long-term stability of printed proteins
- Compatibility with all detection methodologies
- Compatibility with commercially available arraying robots

The nitrocellulose coating binds proteins in a noncovalent and irreversible manner and can be probed using the same method as in traditional blots. The FAST Slide's glass

surface features a matrix that retains arrayed proteins in nearly quantitative fashion, resulting in unparalleled sensitivity down to antigen concentrations of 1 pg/mL. In the arraying buffer, proteins retain binding potential for more than 800 days. FAST Slides are compatible with fluorescent, chemiluminescent or radiographic detection systems and commercially available arraying robots. FAST Slides are suitable for many types of protein microarrays. FAST Slides require a smaller sample and have better sensitivity, linearity and quantitation than traditional ELISAs and Western blots. In addition, thousands of antibodies or samples can be screened simultaneously.



FAST SLIDES

## Product Range

Product	Properties
FAST Slide	Uses proprietary nitrocellulose matrix, providing much larger surface area for protein immobilization
FAST Quant®	Multiplex human cytokine screening, used to quantify up to 10 cytokines simultaneously
Serum Biomarker Chip	Single-capture antibody array to pattern the relative abundance of 120 human serum biomarkers
CombiChip™	Antigen array used to profile research or clinical samples for the presence of autoantibodies (see also page 18)

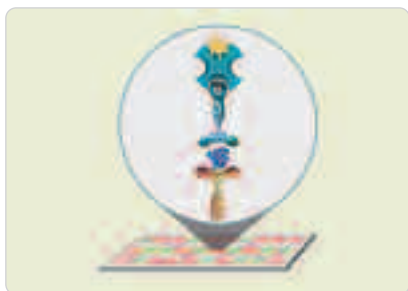
FAST Slide Features	Benefits
3-D proprietary nitrocellulose matrix	Ensures unmatched binding capacity and unparalleled sensitivity of detection in the range of zeptomoles (10 <sup>-21</sup> moles)
Specialized nitrocellulose layer maintains high protein binding capacity and reproducibility	High and reliable signals
Compatible with existing arraying robots, scanners and detection methods	Low setup costs; shortened startup time
Stabilizing environment for proteins when using the proprietary Whatman Arraying Buffer	Long-term stability of protein arrays

# PROTEIN ARRAY SOLUTIONS ON FAST® SLIDES

## ARRAY SOLUTIONS

Our comprehensive product portfolio and exceptional technology present an array of solutions in research and diagnostics.

### Microspot ELISA and Antibody Arrays



Microspot ELISA and antibody arrays are used for quantitative profiling of protein expression in cell cultures and clinical specimens, such as serum and plasma. Typically these arrays are low density (9–100 elements per array). The Whatman FAST Quant® Cytokine Quantification kits offer an example of a microspot sandwich array (go to [www.whatman.com](http://www.whatman.com)).

### Single-Capture Antibody Arrays



Single-capture antibody arrays use direct or hapten labeling systems, which do not require two antigen-specific antibodies. The Whatman Serum Biomarker Chip is an example of a single-capture antibody array.

### Antigen Arrays or Reverse Arrays



One application of antigen arrays is to examine research or clinical samples for the presence of autoantibodies. Normally a low-density array of approximately 10 to 20 antigens can be probed with serum or plasma samples. The Whatman CombiChip™ Autoimmune kit is an example of an antigen array.

Reverse arrays are used to examine dozens to hundreds of research or clinical samples for the presence of one to three antigens. This creates an array of unknowns that can be probed with a small number of antibodies. Visualization can be performed with a detection or top antibody linked to a fluorophore or color detection reagent.

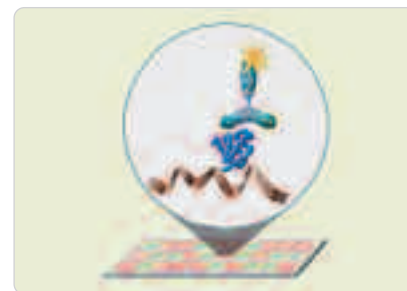
### Proteomic Arrays



Proteomic arrays are typically high-density arrays (>1,000 elements per array) that are used to identify novel enzymes or protein-protein interactions. The arrayed elements can come from any one of many possible sources, including expression libraries, and can contain known and unknown elements. To detect protein interactions,

the samples must be labeled directly with a fluorophore or a hapten. Alternatively, in some applications, antibodies can be used to detect binding events.

### Protein Binder Arrays



Protein arrays can be used to identify novel protein-binding motifs or protein-protein interactions. Engineered or synthetic proteins, or peptides with various binding motifs, are arrayed. The array is then probed with complex protein samples. Detection with a known antibody allows the researcher to identify previously unknown binding events.

# COMBICHIP™ AUTOIMMUNE 1.0

## FOR DIAGNOSIS OF THE AUTOIMMUNE DISEASES VASCULITIS & COLLAGENOSIS

- Simultaneous quantitative analysis of 14 autoantibodies
- Easy as an ELISA test
- Up to 64 samples in one working cycle

CombiChip Autoimmune 1.0 contains a collection of autoantigens that cover the most important autoimmune diseases in the range of collagenosis and vasculitis. For the first time 14 autoantibodies can be proved simultaneously.

### Antigen Array for Multiplex In-Vitro Diagnosis of Human Autoimmune-Antibodies

CombiChip Autoimmune 1.0 is a brand new technology for simultaneously analyzing 14 autoantibodies indicative of

collagenosis and vasculitis. Built on FAST® Slides, the nitrocellulose-coated slides with highest protein-binding capacity, CombiChip Autoimmune combines the power of array technology with the proven surface for Western blot applications and the high-throughput capabilities of traditional ELISA. Each CombiChip slide consists of 16 identical arrays (i.e., 16 identical pads). Each array contains 14 autoantigens and an internal calibrator curve consisting of human immunoglobulin-gamma (IgG).

Up to 16, 32, 48 or 64 patient sera can be analyzed in parallel. For this purpose up to four CombiChip slides are inserted into the FAST Frame slide holder, together with the corresponding incubation chambers. First 70 µL of diluted serum is incubated on each array pad. Then autoantibodies that bind to their specific antigen are detected with an anti-human IgG conjugate containing a fluorescent tag. The internal calibration curve, composed of human IgG, is linear over more than 2.5 orders of magnitude and allows for relative quantitative detection of the autoantibody pattern.

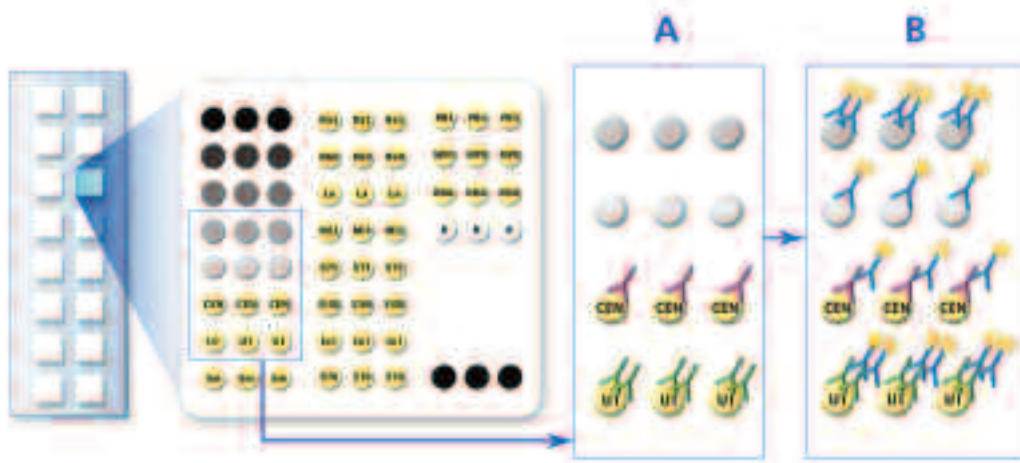


COMBICHIP AUTOIMMUNE 1.0

After processing, the arrays can be imaged using any common microarray scanner and then analyzed with easy-to-use software.

CombiChip Autoimmune 1.0 contains a collection of autoantigens that cover the most important collagenosis and vasculitis autoimmune diseases. For the first time, 14 autoantibodies can be measured simultaneously—on one array pad—and can be used in parallel for a comprehensive, detailed diagnosis.

## Processing of the Array



**70 µl of diluted sample (1:100) is incubated on each array pad.**

Step A	The reactive autoantibodies bind to their specific antigen
Step B	Anti-human IgG conjugate binds to human IgG antibodies, including the calibrator curve consisting of human IgG

CombiChip is then scanned in a fluorescent imager, and data are analyzed.

# CUSTOM PROTEIN ARRAY DEVELOPMENT

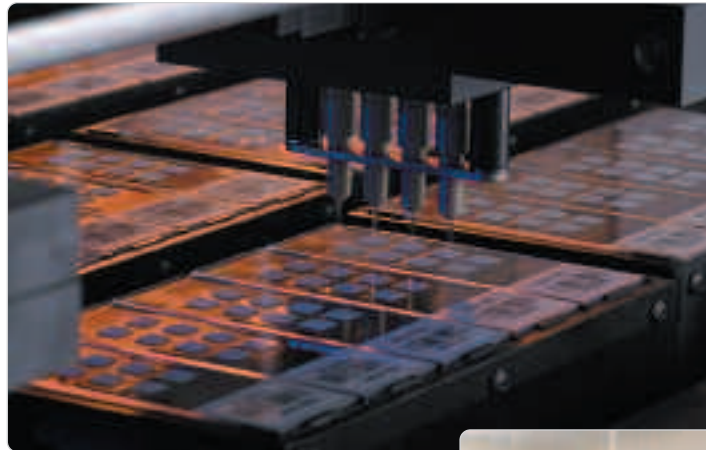
Whatman, the leader in protein array technology, offers a comprehensive group of services ranging from custom development to processing to scanning and data analysis. All services are based on the widely accepted FAST® Slide microarray platform.

## Custom Array Development

The custom array service at Whatman enables scientists with access to microarray scanning and data analysis instrumentation to have FAST Slides printed with proteins from the Whatman antibody menu or from your own protein library. The custom service provides the scientist complete control of proof of principle, assay design, processing and data analysis of the printed array.

## Processing arrays

Using leading-edge automated microarray technologies and innovative scientific approaches, the protein array



PRINTING OF PROTEIN ARRAYS

development, processing and data analysis service allows researchers to acquire distinct reliable scientific data from the proteomic specialists at Whatman. Protein array development at our facility encompasses proof of principle, assay design, array printing, processing, and data analysis.



PROTEIN ARRAY MANUFACTURING AND PROCESSING AT WHATMAN

## Scanning and Data Analysis

Whatman offers a slide scanning and data analysis service for FAST Slide users who do not have access to a fluorescent scanner.

## Custom Array Development Service



## Processing Service

Proteomic specialists at Whatman process customized protein arrays and deliver distinct and reliable data.

## Scanning and Data Analysis

For customers without scanner: FAST Slide scanning and data analysis service for processed Whatman protein array kits.

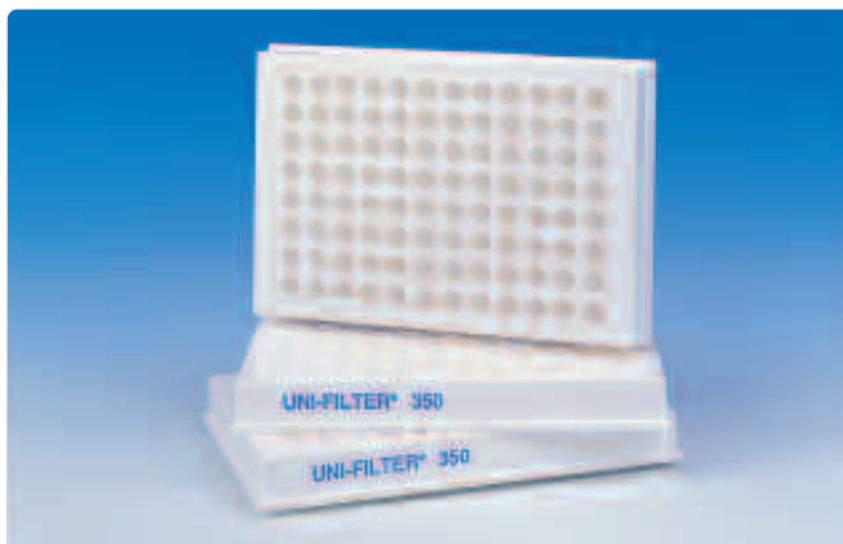
# FILTERPLATE-BASED ELISA

## ELISA

Traditional ELISA is performed in plastic microplates. Filterplate-based ELISA enables faster and more sensitive assays than plastic-based assays. With the binding characteristics of nitrocellulose or polyvinylidenedifluoride (PVDF) membranes in a standard multiwell format, users can significantly increase the amount of capture reagent in each well, therefore significantly increasing sensitivity. Coating the membrane takes just minutes, compared with overnight procedures for polystyrene microplates. The vacuum filtration step greatly reduces the time required for the washing steps.

### Key Features and Benefits

- **Rapid ELISA filterplate.** Assays are complete in less than 30 minutes rather than more than 4 hours, as with traditional plates.
- **Simple filtration steps.** The use of a vacuum filtration step removes the need for expensive well-filling liquid handlers.
- **Fast and easy protocol.** No long overnight immobilization steps required.
- **No well-to-well contamination.** Unique plate design stops samples from migrating between wells.
- **Large surface area.** The ability to bind capture reagents to the membrane can increase the amount of capture reagent binding to 200 times that of plastic plates.



ELISA UNIFILTER

- **Easy waste handling and cleanup.**  
The samples and wash buffers are drawn through the membrane into a collection plate.
- **Compatible with automated platforms and liquid handlers.**  
The plates are manufactured to ANSI/SBS (American National Standards Institute and Society for Biomolecular Science) standards, compatible with most commercial systems.



UNIVAC 3 – FILTER TO COLLECT

Use of a filterplate offers two alternative ways of reading the result of an ELISA. The method to use depends upon the type of plate reader to be used to detect results. It is necessary to use a vacuum manifold with either method; however, which manifold to use depends upon the application. UniVac™ 1 is a very simple vacuum manifold that simply draws the sample through and runs the mixed samples to waste. UniVac 3 allows the samples to be collected in a standard collection plate; typically a 2 mL plate is used. The UniVac 3 also allows control of the vacuum pressure during the filtration steps. For some viscous samples it is important to be able to control the vacuum to minimize splashing and potential cross-contamination of samples in the collection plates.

### Types of ELISA Assay That Can Be Performed in Filterplates

Filterplates contain a membrane that prevents measurement of the signal through the bottom of the plate, as is seen in traditional ELISA assays. The mechanism of reading therefore has to be adjusted to compensate for the difference in design.

For chemiluminescent assays the bottom of the filterplate must be sealed with a plastic film to prevent liquid leaking out. Results are obtained by placing the sealed plate in a traditional chemiluminescent reader. Similarly, a fluorescent assay can be read directly from the plate provided the fluorescent substrate is bound to the membrane. For a traditional ELISA system whose result is indicated by the presence of a soluble color, the solution containing the colored reagents must be filtered through the membrane and collected in a traditional



UNIVAC 1 – FILTER TO WASTE

solid-bottom plate. The color intensity is read by placing the collection plate in a standard plate reader.

It is possible to use all methods with filterplates; however, the best results have been obtained when using chemiluminescent techniques.

Reference Literature:

- Whatman UNIFILTER ELISA Protocol  
Download at [www.whatman.com](http://www.whatman.com)

### Protocol

1. Place ELISA filterplate on vacuum manifold and add 200  $\mu\text{L}$  of PBS pH 7.2. Incubate for 2 minutes, then remove by vacuum.
  - a. For UniVac 3, place a 2 mL collection plate under the filterplate to collect the liquid that passes through. This is not necessary for the UniVac 1 system.
2. Add 50  $\mu\text{L}$  of coating antibody diluted\* in PBS pH 7.2; incubate for at least 5 minutes before removing by vacuum.
3. Dry the plate for a minimum of 30 minutes at room temperature.
4. Add 100  $\mu\text{L}$  of blocking buffer (PBS pH 7.2 + 3% BSA) and incubate for 5 minutes before removing by vacuum.
5. Dilute sample in a standard dilution buffer (PBS pH 7.2 + 1% BSA + 1% Tween 20<sup>®</sup>). Dilution levels will depend upon analyte concentration within the sample.<sup>†</sup> Apply diluted sample, incubate for 15 minutes and remove by vacuum.
6. Wash 4 times by adding 200  $\mu\text{L}$  wash buffer (PBS pH 7.2 + 0.1% Tween 20) with vacuum on.
7. Dilute the detection antibody conjugate in the standard dilution buffer<sup>‡</sup> and add 50  $\mu\text{L}$  to the filterplate. Incubate for 15 minutes and remove by vacuum.
8. Wash 4 times by adding 200  $\mu\text{L}$  wash buffer (PBS pH 7.2 + 0.1% Tween 20) with vacuum on.
9. Add 100  $\mu\text{L}$  of enzyme substrate and develop until color appears.
  - a. For soluble substrate collect the filtrate by vacuum filtration into a 350  $\mu\text{L}$  collection plate (7701-1350) for reading.<sup>§</sup>
  - b. For insoluble substrate wash 4 times by adding 200  $\mu\text{L}$  wash buffer (PBS pH 7.2 + 0.1% Tween 20) with vacuum on and then read the results directly.
  - c. For chemiluminescence seal the plate top and bottom using sealing film and read directly with the substrate in the wells.

#### Notes:

\* The dilution and incubation times required depend upon the particular system. Typical conditions are 10 mg/mL for 5 minutes; however, successful results have been obtained with concentrations as low as 0.5 mg/mL. Incubation time can vary between 5 and 30 minutes depending upon the antibody concentration.

<sup>†</sup> Exact dilution will depend upon analyte concentration. For initial studies use a serial dilution between 1:2 and 1:1000.

<sup>‡</sup> Exact dilution will depend upon analyte concentration. For initial studies use a serial dilution between 1:2 and 1:100.

<sup>§</sup> It is necessary to use plate 7701-1800 as a spacer to ensure correct positioning of the collection plate within the UniVac 3. One spacer plate is included with each UniVac 3. Additional plates can be ordered.

# DIPSTICK/COLOMETRIC ASSAYS

## CELLULOSE MATERIALS FOR DIPSTICK ASSAYS

The use of a dipstick colorimetric assay, in which a cellulose pad is impregnated with a color reagent, is very familiar. They are widely used in everything from urine testing to environmental assays. The base cellulose is a key part of the system, and the correct choice of absorbance, wicking rate and wet strength are critical to producing a working assay.

The Whatman range of cellulose materials for colorimetric assays offers the most consistent and inert substrate for absorption of the active chemicals required for development of dipstick

tests. The purity of the cellulose base material coupled with Whatman quality manufacturing practices makes these papers the ideal choice for large-scale manufacturing.

The Whatman range of papers also includes a wet-strengthened grade. CF2 contains an FDA-approved resin that binds the cellulose fibers together. This significantly increases the strength of the paper, especially when it is wet. The use of the wet-strengthened grade therefore reduces waste during manufacture (due to snapping of the cellulose during coating) and also reduces the risk of the pad decomposing during use.



### Typical Test Data

Grade	Thickness ( $\mu\text{m}$ @ 53 kPa)	Water Absorption ( $\text{mg}/\text{cm}^2$ )
CF2	172	16
CF1	176	16
23SL	250	36
CF3	322	31
CF4	482	46
CF10	490	42
470	840	78
CF5	954	98
CF7	1873	198



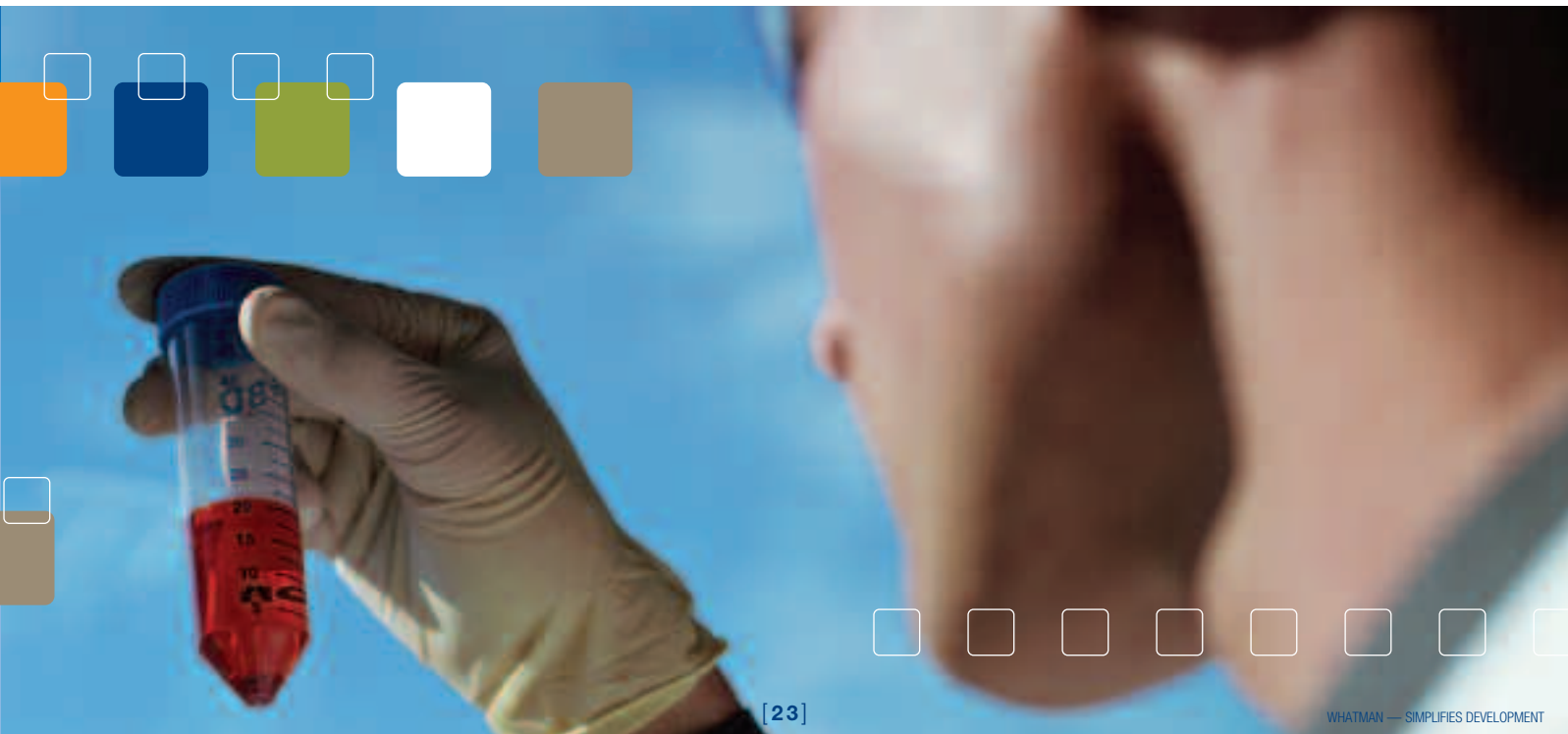
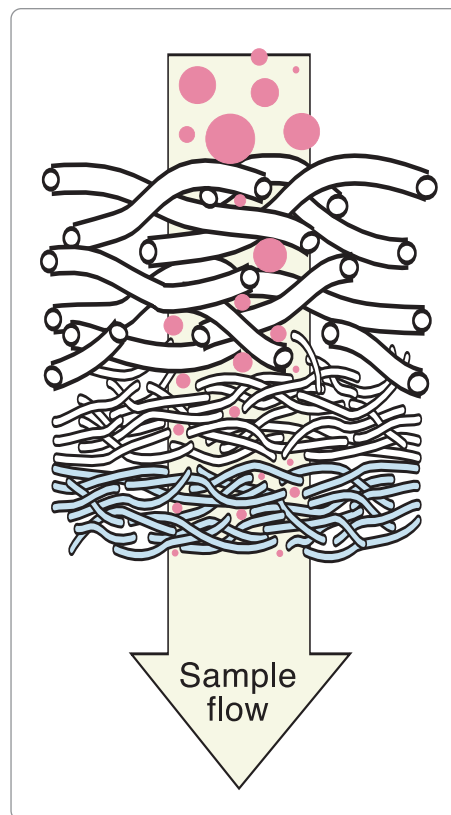
# INTRODUCTION TO SAMPLE PREPARATION

## SIMPLIFY SAMPLE PREPARATION

Today's modern analytical techniques offer the opportunity to achieve extremely high levels of sensitivity. However, to benefit from the increased sensitivity offered by these techniques, significant improvements in sample preparation are necessary.

These improvements in sample preparation prior to the assay will result in reduction of both false-positive signals and the amount of the analyte or target cells used in the assay.

The use of filtration-based techniques offers major advantages. The use of a filter in a simple housing or plate format allows very rapid and cost-effective sample preparation. Filtration-based sample preparation has been shown to work with both traditional protein-based assays and molecular diagnostic applications. The choice of membrane devices influences the ability to remove red cells to stop interference by heme and to stop problems caused by contaminants. In molecular diagnostics the removal of host DNA by removal of white cells has proved to be a very effective way of improving the detection limit for some assays. In a particular example, use of a filtration material that removed white blood cells produced a 50-fold increase in sensitivity on a PCR-based malaria assay.



# TRACK-ETCHED MEMBRANES

## HIGHLY CONTROLLED PORE SIZE

Track-etched membranes (TEMs) are very thin, with tightly controlled pore sizes. They have traditionally been used for high-specification filtration in many laboratory applications. These thin films have discrete pores that are formed through a combination of charged particle bombardment (or irradiation) and chemical etching.

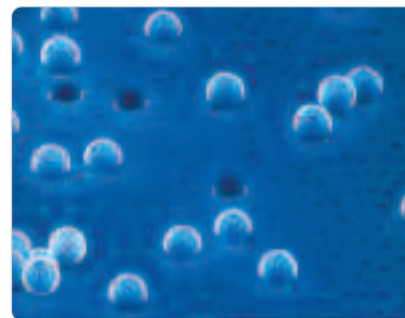
### Properties of TEMs

TEMs exhibit a number of properties that give the material a unique overall performance. Exploiting different combinations of these properties has allowed the development of applications offering advantages that other membranes may not provide.

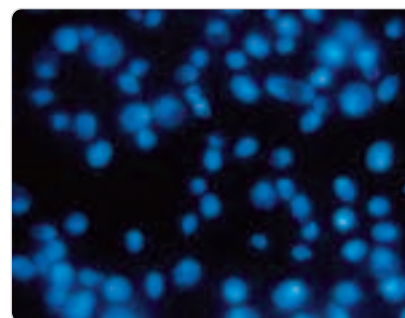
- **Optical Properties.** TEMs are usually transparent when the pore size is larger than 5 µm. Membranes with pore sizes smaller than 3 µm are opaque, although

they can be made transparent in a carefully controlled manufacturing procedure. The membrane can also be dyed if light transmission is undesirable.

- **Controlled Thickness.** The polymeric films used for TEMs (PC or PET) are inherently smooth. Thickness variations in TEMs are kept within ±1 µm; surface variation does not exceed 50 nm (peak to valley).
- **Pore Size and Distribution.** The pore size of TEMs can vary from approximately 20 nm to 14 µm. TEMs also have very controlled pore size distribution because of the manufacturing process. In addition to pore size, pore density (or porosity) can be controlled. It typically ranges from  $1 \times 10^5$  to  $6 \times 10^8$  pores/cm<sup>2</sup>.
- **Low Reactivity.** TEMs are manufactured commercially in either PC or PET polymers, neither of which reacts with biological materials.
- **Low Liquid Diffusion Rate.** Because of the low porosity of the membrane, the flow rate through the membrane is very low for materials with small pores. This flow rate can be controlled by the choice of pore size and pore density.



LATEX BEADS ON CYCLOPORE® MEMBRANE



YEAST CELLS ON BLACK CYCLOPORE WITH DAPI STAIN

Features	Benefits
Biologically inert	Whole cell assays can be performed
Low protein binding and low extractables	There is no interference with assay results because of the membrane chosen
Choice of surface properties (hydrophilic and hydrophobic versions available)	Assays can be designed with the appropriate flow or retention characteristics
Does not bind stains or labels	Gives lower background signal than traditional materials
True surface capture on a flat, smooth surface	Cells or particles are highly visible or available for simple recovery by backflushing
Low hold, up volume	Practically all of the applied sample is available for analysis
Controllable optical properties (transparent, translucent and dyed)	The optical properties can be chosen to ensure optimal signal-to-noise ratio. Clear materials allow complete transmission of light, whereas dyed varieties block signals from behind the membrane.
PC or PET material	Allows easy attachment to a range of housings for design of components

## APPLICATIONS OF TEMs FOR IN VITRO DIAGNOSTICS (IVDs)

In a number of areas, the combination of properties associated with TEMs makes them suitable for use in IVDs.

- **Flow Controller.** The combination of pore size and pore density gives a membrane its flow characteristics. With accurate control of these properties, a smooth surface and good thickness control, TEMs are suitable for applications in which the flow rate of a liquid through the membrane must be controlled. A TEM's ability to control flow rate is particularly applicable in biosensors, when liquids must be in contact with the sensor for a certain time, as well as in flow-through assays and lateral-flow tests.
- **Cell Capture.** Since TEMs have tightly controlled filtration characteristics, they can be used in cell capture applications. This application allows for clearer identification of marked cells in a number of formats. The retention of cells upon the membrane surface allows cells to be stained and observed in a very clear environment. Any sample debris or contaminants can be washed through the pores of the membrane

away from the viewing area. The improved resolution and accuracy have applications in any area of clinical chemistry in which cells are observed. The reduction in the likelihood of a false diagnosis also has a significant impact, especially in large-scale screening procedures.

- **Particle-Capture Assays.** Using membranes for particle capture tests is a relatively well-known technique. The utility of these assays can be enhanced by use of a dyed or fluorescent latex particle as a label. These particles enable the production of a more sensitive or stable assay. Using a TEM for particle capture allows for a more specific capture reaction, and capture of the particles on the membrane surface rather than in the depth of a membrane matrix enhances sensitivity.
- **Biosensors.** TEMs provide accurate flow control or diffusion properties in biosensor applications in which the membrane acts as a barrier to biological molecules and controls their flow to the sensor. The membrane also serves as a barrier to many potential contaminants, improving the assay's specificity. In applications involving the presence of biochemical reagents to measure the reaction, the pores can be filled with the desired materials (e.g., antigen or enzymes). The complete biosensor can therefore be dried onto the membrane.



AN ERYTHROCYTE PASSING THROUGH A 2.5  $\mu\text{m}$  PORE IN A TRACK-ETCHED MEMBRANE

- **Erythrocyte Deformability Testing.** Healthy erythrocytes are highly flexible molecules that will readily change their shape to pass through small openings. In some diseases, such as sickle cell anemia, diabetes, and some cardiovascular conditions, the cells become rigid and can no longer pass through small pores. Healthy red cells are typically 7.5  $\mu\text{m}$  and will easily pass through a 3  $\mu\text{m}$  pore membrane, whereas a cell with one of these disease states will not. In the deformability test, a 5  $\mu\text{m}$  membrane is used as a screening barrier. A blood sample is applied and the membrane is placed under a constant vacuum. The filtration rate of the cells is then measured, and a decreased rate of filtration indicates decreased deformability.

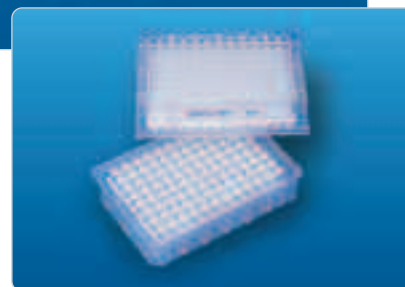
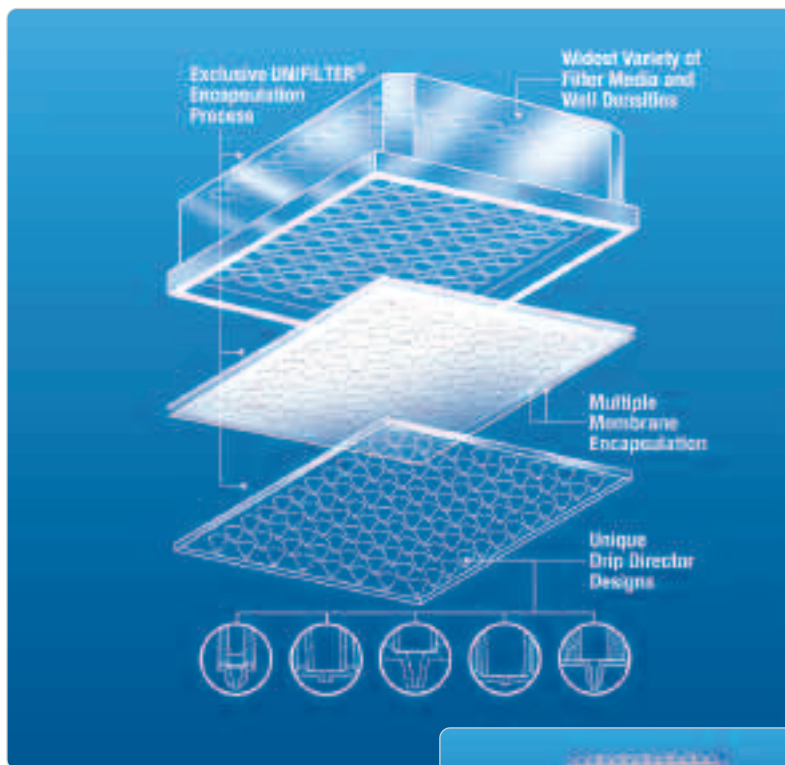
# ENCAPSULATION CAPABILITIES

## PROVEN WHATMAN MEDIA IN DEVICE FORMAT

Whatman can encapsulate filter media into various formats, such as microplate, syringe filter and centrifuge tube filter. The wide range of devices can meet the sample volume and throughput you require.

### UNIFILTER® Filtration Microplates

Whatman UNIFILTER microplates allow faster high-throughput sample preparations for assays such as ELISA. The multiwell format also lets you significantly lower the cost of your assay per sample. Proprietary welding technology prevents crosstalk or well-to-well cross-contamination.



Well Format and Volume	
Well Format	Well Volume
384	100 µL
96	150 µL, 350 µL, 800 µL, 2 mL
24	10 mL

Drip Director	
Director Type	Filtration Process
Mesh	Filter to waste
Short	Filter to waste with vacuum or filter to collect with centrifuge
Long	Filter to collect with vacuum or centrifuge
Closed	Filtrate not going through filterplate

Filterplate Application Guide	
Product	Use
VF4	Removes particles greater than 0.7 µm, e.g., some bacteria
VF3	Removes particles greater than 1.2 µm, e.g., yeast cells
MF1	Removes particles larger than 2 µm with moderate loading capacity, e.g., red cells and platelets
VF1	Removes particles larger than 2.5 µm with high loading capacity, e.g., red cells
VF2	Removes particles larger than 3 µm, e.g., mammalian cells
VFE	Removes particles larger than 5 µm, e.g., human white cells or epithelial cells
Nitrocellulose 0.45 µm	For binding of proteins (> 20k MW) and DNA for ELISA-type assays
PVDF 0.2 µm (hydrophobic)	For binding of all types of proteins in ELISA assays
Polypropylene mesh (hydrophilic)	For rapid removal of large particles and crude filtration of samples



PLATE CONFIGURATIONS

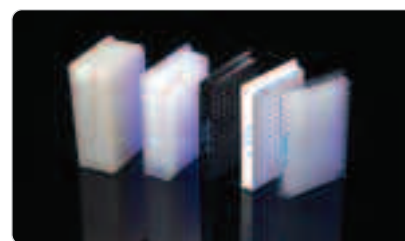


PLATE COLORS

### Key Features and Benefits

- **The filter is cut and sealed in each well.** Eliminates contamination worries even with viscous samples.
- **Broad range of filter media.** Includes glass fiber, polypropylene, cellulose nitrate, nylon, PVDF, cellulose acetate and ion exchange cellulose.
- **Complies with ANSI/SBS standard.** Easy to automate.
- **Low sample hold-up.** Reduces sample size and gives higher sensitivity.



### Plate Materials

- **Clear Polystyrene.** Well contents can be seen.
- **White Polystyrene.** Suitable for chemiluminescence and radioactivity.
- **Black Polystyrene.** Suitable for fluorescence.
- **Natural Polypropylene.** Semi-clear. Chemically resistant.
- **Glass-filled Polypropylene.** Better chemical resistance than natural polypropylene.

### Applications

- Sample preparation
- Blood separation
- Bead assay
- ELISA assay
- ELISPOT assay
- Nucleic acid purification

### Accessories

Whatman provides a range of accessories to meet your needs, such as vacuum manifolds, lids, seals and cap mats. For complete details, please contact your local representative or visit [www.whatman.com](http://www.whatman.com).



### Syringe Filters

Whatman offers a complete line of disposable syringe filter devices to provide fast and efficient sample preparation. Whatman syringe filters are composed of a pure polypropylene housing, heat sealed without the use of glues or sealant.

Syringe filters are available in 4, 13, 25, and 30 mm diameters. The diameter chosen depends upon the volume of sample to be filtered.



25 mm SYRINGE FILTER



4 mm SYRINGE FILTER



13 mm SYRINGE FILTER WITH TUBE TIP

# GLASS FIBER FILTERS FOR SAMPLE PREPARATION

Many assays require that any contaminants be removed from samples prior to application. Proper sample preparation can improve detection sensitivity and decrease processing time. Depending on the sample type and specific components to be removed, any of several materials can be used. Whatman offers a full range of proven high-quality sample preparation technologies.

## Key Features and Benefits

- **High flow rates and loading capacity.** Whatman glass fiber materials provide superior performance compared with fine-pore membranes.
- **Selective particulate removal.** Whatman filters enable removal of particles of known diameters.
- **Guaranteed performance.** Whatman glass fiber is the recognized gold standard for particle filtration.

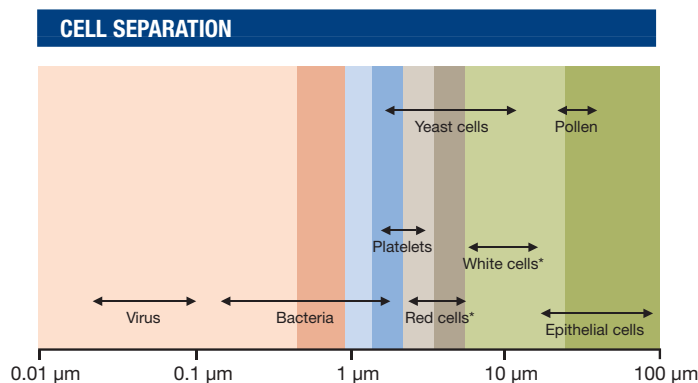
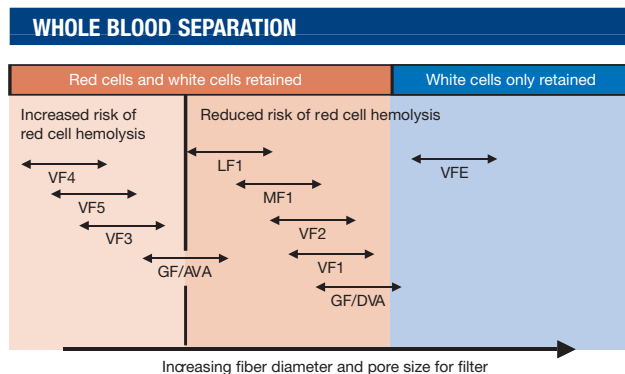


VFE UNIFILTER®

## VFE UNIFILTER®

In many applications, the sensitivity of an assay is limited by the presence of large amounts of host nucleic acids. These nucleic acids, contained in cells such as white or epithelial cells, significantly reduce the sensitivity of an assay. Through a

simple filtration step, Whatman VFE UNIFILTER removes these host cells, making PCR-based assays up to 50 times more sensitive.



For example, to remove white cells but let red cells go through, choose grade VFE.  
 \* This is the effective radius for human cells, as the cells can deform when passing through smaller pores.

Recommended Grades	
Use membranes	VF1
VF4	VF2
VF3	VFE
MF1	Polypropylene mesh

## Product Range

Grade	Particle Retention* Liquid (µm)	Binder	Use
VF4	0.7	None	Removes particles greater than 0.7 µm, e.g., some bacteria
VF3	1.2	None	Removes particles greater than 1.2 µm, e.g., yeast cells
VF5	1.0	None	Removes particles >1 µm but with higher loading capacity than VF3 or VF4
GF/AVA	1.7	Polyvinyl alcohol-bound	Stronger than binder-free glass fiber filters
MF1	2	None	Removes particles larger than 2 µm with moderate loading capacity, e.g., red cells and platelets
VF1	2.5	None	Removes particles larger than 2.5 µm with high loading capacity, e.g., red cells
VF2	3	None	Removes particles larger than 3 µm, e.g., mammalian cells
GF/DVA	3.5	Polyvinyl alcohol-bound	High loading capacity; rapid filtration and removal of coarse particles
VFE	5	None	Removes particles larger than 5 µm, e.g., human white cells or epithelial cells

\* 98% particle retention rating.

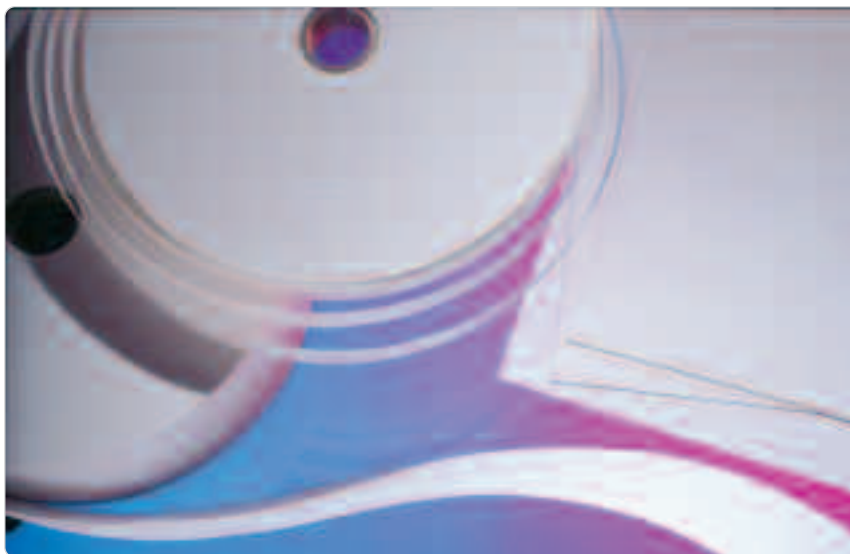
# SAMPLE COLLECTION

## FOR ACCURATE AND RELIABLE SAMPLE COLLECTION

To ensure minimal sample interference, Whatman uses only high-purity raw materials in its manufacture of cellulose papers. The wide range of papers available can be used to collect and transport bodily fluid samples including blood, saliva, urine, sweat, plasma, tears and feces.

### Key Features and Benefits

- **Minimal sample interference.**  
High-purity raw materials are used in the manufacturing process.
- **Extensive collection capabilities.**  
Wide range of absorbencies can accommodate varying sample types.
- **Easy sample transportation.**  
Samples can be dried on the material for subsequent rehydration.



## Product Range

Product	Property
CF2	Suitable for collecting small samples of semisolid matter; incorporates wet-strength binder for improved strength during sample application
CF3	100% cotton linter; medium absorbency for collecting samples and high purity requirements
CF5	100% cotton linter; higher absorbency than CF3; suitable for larger sample volumes
CF7	More absorbent 100% cotton linter material; ideal for saliva and other large-volume sample collections
CF10	High-purity cotton linter material; most suitable for assays to detect proteins or enzymes
903 Proteinsaver	High-purity cotton linter material with no additives. Suitable for assays to detect proteins, enzymes or metabolites. Widely used as a sample collection device for biological specimens.

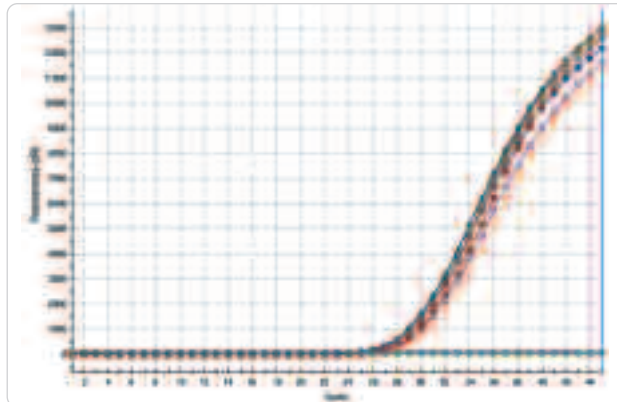
## Typical Test Data

Grade	Thickness ( $\mu\text{m}$ @ 53 kPa)	Wicking Rate (s/4 cm)	Water Absorption ( $\text{mg}/\text{cm}^2$ )
CF2	172	187	16
CF3	322	161	31
CF10	490	92	42
CF5	954	75	98
CF7	1873	35	198

## COLLECTION METHODS



## REAL-TIME PCR



Ten individual samples of DNA purified using FTA Elute were prepared for real-time PCR of an in-house reference DNA fragment. For this assay 2.5  $\mu\text{L}$  of the extracted DNA was mixed with the DNA binding dye Yo-Pro 1 (Invitrogen) to monitor real-time PCR amplification. A no-template control included in the assay appears as the flat line at the bottom of the profile.

Blood samples were collected from 10 separate individuals onto FTA<sup>®</sup> Elute cards, and the cards were allowed to dry completely. Punches (3.0 mm) were extracted according to the water and heat elution protocol in a final volume of 100  $\mu\text{L}$ . Approximately 2.5  $\mu\text{L}$  of the purified DNA was added to the real-time PCR mixture and amplified using the Yo-Pro 1<sup>®</sup> DNA binding dye (Invitrogen). The figure above shows a very tight grouping of curves with an average Ct of 26.58, which equals 22.14 ng of DNA in the 100- $\mu\text{L}$  final volume. Considering that the samples are from 10 individuals, any

variation in the Ct values are due to differences among individuals (as would be seen in a white blood cell count). The data is highly reproducible from the 10 individual samples, which shows the high quality of DNA purified using FTA Elute. The yield of DNA from FTA Elute is sample dependent. For blood, over a large number of determinations, yield ranges are 55–70%. For buccal samples, over a large number of determinations, yield is generally higher, 60–75%. The easy extraction procedure also demonstrates that the DNA obtained is amenable to high-throughput genotyping.

## MULTIPLEX GENE DELETION ASSAY



Amplicons from a multiplex PCR were separated on a 1.5% agarose gel. Lanes 1–10 are reactions using DNA from 10 separate individuals. The control lanes demonstrate the possible genotypes: A, wt/wt; B, wt/mut; C, mut/mut. The Abgene Electrofast MWMarker Ladder: 250 bp, 500 bp, 1000 bp is in Lanes M.

Many labs perform multiplex gene deletion assays using end point PCR and agarose gel electrophoresis as a means of genotyping. The presence or absence of bands for a large deletion is clearly visible on the gels. The figure above shows a multiplex PCR performed with DNA purified from FTA Elute to detect a deletion in the UGT2B17 gene. The wild-type and mutant genes are demonstrated by bands of 316 bp and 884 bp,

respectively. As can be seen, the individual in lane 7 is homozygous for the mutant form of the gene.

The ability to multiplex with multiple primers depends on high-quality DNA to prevent mispriming and nonspecific banding patterns. The DNA purified by FTA Elute is very robust, and less than 1 ng is required to yield clean, strong bands in the multiplex PCR.

## Whatman Quality

Whatman is a global leader in separations technology and is known in the scientific community for providing innovative life science products and solutions. Our instinct for simplification accelerates the rate of discovery, reduces costs and saves time. For more information, visit [www.whatman.com](http://www.whatman.com)

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Yo-Pro 1® is a registered trademark of Invitrogen.

For specialized technical support departments, E-mail [diagnostics@whatman.com](mailto:diagnostics@whatman.com).

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