

Filterplate for Standard ELISA

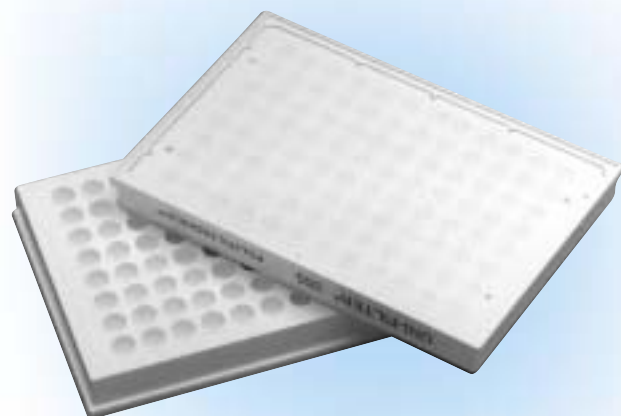
Enzyme-Linked Immunosorbent Assay (ELISA) is a standard methodology capable of quantitating an antibody or antigen of interest with a very high degree of accuracy and sensitivity. There are four basic steps involved in the technique:

1. Immobilizing a ligand to a solid support
2. Binding of a sample analyte (antibody or antigen) to the immobilized ligand
3. Attachment of a conjugated analyte to the sample analyte
4. Detection

Heterogeneous assays such as ELISA involve the separation of free analyte from the bound analyte so that the sample may be quantified. Performing this separation via filtration offers several advantages over commonly used plastic 96-well microplates such as speed and higher available binding capacities.

ELISA performed with the Whatman nitrocellulose filterplate takes less time than traditional methods using regular microplates for the separation. Coating the nitrocellulose filter takes only minutes, compared with overnight procedures employed for coating polystyrene microplates. Also, the use of vacuum filtration greatly reduces the time required for washing steps, and enables quantitative collection of filtrate into a collection plate.

The Whatman ELISA filterplate allows researchers to utilize the excellent protein binding characteristics of nitrocellulose (~40µg IgG per well) in a standard SBS 8 x 12 multiwell format. As well as ELISA, the Whatman nitrocellulose filterplate can also be used for other types of ligand binding applications including hybridoma screening. It is totally compatible with automated systems, as well as the Whatman filter-to-collect vacuum manifold and filtrate collection microplates.



Features

- 96 well, 350µL per well, 8 x 12 format constructed from opaque white polystyrene, which is chemiluminescent compatible
- Individual discrete disks of Whatman Nitrocellulose (0.45µm) at the bottom of each well
- Patented drip director design for the non-crosstalk collection of filtrate
- Compatible with many automated platforms and liquid handlers

Benefits

- High precision, accuracy and reproducibility
- Sensitivity comparable to standard microplates
- Fast and easy protocol—no long overnight immobilization steps required
- Adaptable to other separations based assays

Protocol

1. Place a 2mL Waste Collection Plate in the bottom of the vacuum manifold
2. Place ELISA filterplate on vacuum manifold and prewet the membrane with 200µL PBS. Incubate 2 minutes, then remove by vacuum
3. Add 50µL of coating antibody (10µg/mL) in PBS, incubate 5 minutes, and remove by vacuum
4. Add 100µL of 1 - 3% BSA in PBS blocking buffer. Incubate 5 minutes and remove by vacuum
5. Apply sample diluted in 1 - 3% BSA, 0.1% Tween in PBS. Incubate 15 minutes and remove by vacuum
6. Wash 4 times with 200µL 0.1% Tween in PBS on the manifold
7. Empty the Waste Collection Plate
8. Add 50µL of the conjugated antibody diluted in 1% BSA, 0.1% Tween in PBS. Incubate for 15 minutes and remove by vacuum
9. Wash 4 times with 200µL 0.1% Tween in PBS on the manifold
10. Remove the Waste Collection Plate from the bottom of the vacuum manifold and replace with the 300µL Collection Plate on top of an 800µL Stacker Plate
11. Add 100µL of conjugated enzyme substrate and incubate until color develops
12. Apply vacuum to collect filtrate into the Collection Plate. Place in plate reader

Note: If using chemiluminescent detection, the ELISA plate can be sealed and read directly in a plate reader. The final vacuum step is for colorimetric detection methods only.

Products Required

Cat No.	Description	Units/Case
7700-3307	ELISA 96-well Nitrocellulose Filterplate	50
7701-1350	300µL clear Polystyrene 96-well Collection Plate	50
7701-5200	2mL Waste Collection Plate	25
7701-1800	800µL Stacker Plate	25
7705-0107	UniVac™ Vacuum to Collect Manifold, acrylic	1
7704-0001	Clear Polyester Thin Cold Sealing Film Adhesive Backing	100

Whatman®

North America: Whatman Inc. 9 Bridewell Place Clifton, NJ 07014 • Technical Support: 1-800-922-0361 • Customer Service: 1-800-631-7290 • Outside US: 973-773-5800 • Fax: 973-773-0168 • E-mail: info@whatman.com
Europe: Whatman International Ltd Whatman House St Leonard's Road 20/20 Maidstone Kent ME16 0LS England • Tel: +44 (0)1622 676670 • Fax: +44 (0)1622 677011 • E-mail: information@whatman.co.uk
Japan: Whatman Japan KK Daiwa Ueno Building 1F 6-10 Ueno 5-chome Taito-ku Tokyo 110-0005 Japan • Tel: +81 (0)3 3832 6707 • Fax: +81 (0)3 3832 6457 • E-mail: japaninfo@whatman.co.jp
Asia Pacific: Whatman Asia Pacific Pte Ltd 171 Chin Swee Road #08-01 San Centre Singapore 169877 • Tel: +65 6534 0138 • Fax: +65 6534 2166 • E-mail: wap@whatman.com.sg