

Whatman® FUSION 5™ Test Strip Protocol

Dispensing T & C Lines, Spraying and Drying
of Protein A Gold Conjugate for Human
IgG Assay on FUSION 5 Membrane



Items Needed

NAME	SUGGESTED MANUFACTURER
FUSION 5 Membrane	Whatman
60 mm backing	G&L
Latex-Protein A @ 20 mg/mL	DCN
Latex-Mouse IgG @ 20 mg/mL	DCN
Cellulose grade 470	Whatman
Protein A Gold Conjugate, OD=20.0	DCN
Goat Anti-Mouse Gold Conjugate, OD=10.0	DCN
Sucrose	Sigma
Trehalose	Sigma

Equipment Needed

- BioDot AD5000 with 100 µm Ceramic Tip, Vacuum Pump and Wash Station
- Probe Sonicator
- Mini-Vortexer
- 96-well Source Plate
- XYZ3050 with AirJetQuanti3000 Dispenser
- Drying Oven
- BioDot CM 4000 Cutter
- Slitter or Paper Cutter
- 5 mm Cassettes
- Rocker
- Desiccant
- Foil Pouch(es)

Test Card/Strip Preparation

1. Record the details of the batch to the left.
2. Cut FUSION 5 membrane to 44 mm strip(s).
3. Laminate a 44 mm strip of FUSION 5 membrane onto the backing card ensuring that it is aligned with the bottom edge of the card.
4. Turn on AD5000 and computer.
5. Sonicate the test and control line reagents for 1 minute each. Vortex. Pipette each reagent into the appropriate source well of the 96-well plate. Each card will use about 200 µL of each reagent.
6. Add 20% w/v Sucrose and 5% w/v Trehalose to both the Protein A gold conjugate at OD = 20.0 and the Goat anti-mouse gold conjugate at OD = 10.0. Place on rocker until the sugars are completely dissolved.
7. Spray the Protein A gold conjugate at OD = 20.0 at a rate of 10 µL/cm, psi ~1, micrometer opening = 1.0. Perform 1 overspray again at 10 µL/cm.
8. Dry card(s) for 1 hour at 37°C.
9. Spray the Goat anti-mouse gold conjugate at OD = 10.0 at a rate of 10 µL/cm, psi ~1, micrometer opening = 1.0. Perform 1 overspray again at 10 µL/cm.
10. Dry card(s) for 1 hour at 37° C.
11. Cut the cellulose Grade 470 into 18 mm strip(s) and laminate it so that it is aligned with the top of the card.

(continued on reverse)

12. Because of line ramping issues, mark off the first 15 mm of each line. Orient the card so that the wick is on top. Align a ruler on the wick so that '0' is on the left side of the card. Mark off 15 mm lines starting at these points: 58 mm, 115 mm, 173 mm, and 231 mm. Discard these sections after cutting the card into strips with cutter.
13. Cut card into 5 mm strips.
14. Store in a heat-sealed, desiccated foil pouch until ready to test.

Affix Copy Label with the Relevant Information

Product

Batch Number

Optimal Coating Concentration

Date

Sign _____

Date _____

Material Specifications

Sample/conjugate pad/membrane	FUSION 5, width = 44 mm, Whatman
Test line reagent	Latex-Protein A @ 20 mg/mL & 2.016 µL/cm, DCN
Control line reagent	Latex-Mouse IgG @ 20 mg/mL & 2.016 µL/cm, DCN
Gold conjugates	Protein A Gold Conjugate, OD = 20.0, DCN Goat Anti-Mouse Gold Conjugate, OD = 10.0, DCN
Top pad	Cellulose 470, width = 18 mm, Whatman
Backing card	60 mm Backing, G&L
Cassettes	5 mm Cassettes, DCN

Items Needed

NAME	SUGGESTED MANUFACTURER	PART NUMBER
Protein A Gold Conjugate, OD=20.0	DCN	PACO-010
Goat Anti-Mouse Gold Conjugate, OD=10.0	DCN	PACG-010
Sucrose	Sigma	S-9878
Trehalose	Sigma	T-5251
1xPBS	Sigma	P-3813
Tween 20	Sigma	P-1379

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