CYTODIAGNOSTICS

PRODUCT DATA SHEET

Miriad™ RVF Antigen Detection Kit

Description

Miriad™ RVF Antigen Detection Kit contains all of the materials required to create a rapid analytical tool for the detection of an antigen in a sample.

To detect an antigen using this kit a capture antibody against the antigen of interest is spotted onto the nitrocellulose membrane integrated within the specially designed plastic test cartridges supplied with the kit. When sample is applied the antibody spotted on the membrane will capture any antigen present. Successfully captured antigen is subsequently detected through the addition of gold nanoparticles conjugated to a detection antibody specific to the bound antigen. If antigen is present a red coloured dot will appear on the membrane, see figure 1 below.

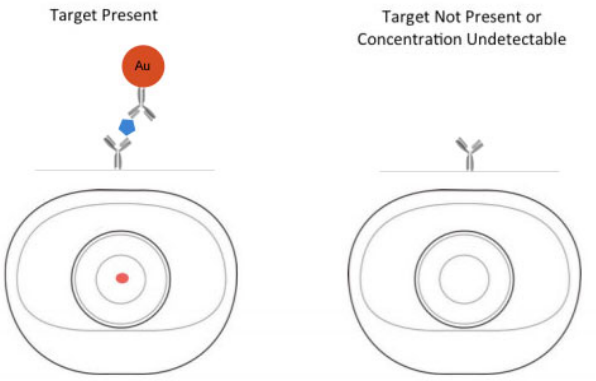

Figure 1. *Illustration of the appearance of the Miriad™ cartridge after application of a gold nanoparticle conjugate (conjugated to an antibody directed toward the antigen of interest) to the membrane when target is present (left), or not present (right).*

In a fully optimized system, the limit of detection of this technology is down to 1 ng/ml of antigen in a particulate free sample. However, iterative optimization may be required to achieve this level of performance and is dependent upon factors such as affinity of antibodies used and type of sampl

Kit Components

- 10 Miriad™Test Cartridges
- 12 ml of Universal Buffer for Miriad™Test Cartridges
- Lyophilized 20nm Gold Nanoparticles (OD=25 if resuspended to 1 ml)
- Protein Resuspension Buffer
- Reaction Buffer
- Quencher

Storage

Store test cartridges and the Universal Buffer for Miriad™ Test Cartridge at room temperature and all other kit component at -20°C. All components stable for at least 1 year if stored as specified.

Product Safety and Handling

This product is for R&D use only, not for drug, household, or other uses. Please review the material safety datasheet (MSDS) available online for proper safety and handling procedures.

Factors to Consider Prior to Conjugation

The Protein/Antibody or other ligand to be conjugated needs to be in a purified form, and proper care must be taken to ensure that the ligand stock is devoid of the following for proper functionality:

- No additional protein additives such as BSA
- Avoid free amino acids (e.g. glycine)
- Avoid common thiol additives such as DTT, TCEP and mercaptoethanol
- Avoid EDTA
- Avoid primary amine containing buffers or components (e.g. Tris)
- Avoid use of strong buffers that might change the pH of the conjugation reaction. See paragraph below for recommended buffers for optimal performance of the kit.

If your protein/antibody stock contains any of the above, dialyse or use a desalting column to transfer your ligand into a compatible buffer such as sodium phosphate, MES, MOPS or HEPES. If contaminating proteins such as BSA is present, the protein needs to be purified prior to conjugation.

I. Conjugation of Detection Antibody to Gold Nanoparticles

A recommended starting protocol for conjugation of a primary antibody to your antigen of interest can be found below. Note that the amount of protein added may need to be optimized for your particular protein.

1. Allow all reagents to warm to room temperature before use.

2. Dilute (or dissolve) your primary antibody to a final concentration of 1 mg/ml using the supplied protein re-suspension buffer.

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3. In a 1.5 ml microcentrifuge tube combine your diluted antibody from step 2 above and the reaction buffer according to the table below.

	Antibody Mix
Reaction Buffer	600 µl
Diluted Antibody Solution	480 µl
Total Volume	1080 µl

4. Transfer 900 µl of antibody mixture to the vial containing lyophilized NHS-activated gold nanoparticles. Immediately mix well by pipetting up and down.

   Note: *Do not resuspend the lyophilized NHS-activated nanoparticles in buffer prior to addition of protein. NHS rapidly hydrolyzes in aqueous solution and may result in loss of conjugation efficiency.*

5. Incubate the vial at room temperature for at least 2 hours.

6. Add 100 µl of quencher solution to the vial to stop the reaction.

7. Using a microcentrifuge, centrifuge the vial with gold nanoparticles for 30 minutes at 5,500 *x g* to pellet the conjugated nanoparticles.

8. Remove the supernatant containing unbound protein.

9. Add 0.5 ml of gold conjugate storage buffer* to the vial to re-suspend your detection probe.

**** Note:*** *A gold conjugate storage buffer is not supplied with the kit. A recommended storage buffer for an antibody gold conjugate and for use with the MedMira cartridge is 20mM Tris (pH 8.0), 150mM NaCl supplemented with 1% (w/v) BSA and 0.025% (w/v) Tween 20. The inclusion of Tween 20 in the storage buffer is imperative to reduce background in the cartridge.*

10. Record the UV-VIS spectra of the conjugate using a spectrophotometer.
11. Dilute the conjugate to an optical density of 10 (at lambda max) using gold conjugate storage buffer.

****Note:** The absorption should be close to 520nm.

12. Store your probes at 4°C until use.

II. Detection of an Antigen in a Sample (Assay Procedure)

II.I Capture Antibody Immobilization Procedure

1. Dilute the desired capture antibody against your antigen of interest to a working concentration using a spotting buffer (1X PBS, pH7.4). Typical protein concentration range is in the order of 1 to 2 mg/ml.

2. Pipette the desired amount of the diluted capture antibody, typically between 0.5 and 1.0 µL, and dispense anywhere on the membrane found in the center of the provided test cartridge.

   Caution: *Care should be taken to avoid pressing the pipette tip into the membrane surface and the membrane surface should not be touched.*

3. The antibody spot needs to dry completely for a minimum of 30 minutes before testing, however, drying times of up to 24 hours may result in a higher protein binding. Alternatively, drying can be enhanced by blowing warm air in a circular motion indirectly towards the membrane using a blow dryer set at low/medium heat for approximately 5 minutes.

4. The prepared cartridges can be stored at room temperature in a dry container with desiccants for a period of up to 12 months, depending upon the nature of the antibody applied.

II.II Testing Procedure for Detection of an Antigen in a Sample

1. Apply 3 drops of Universal Buffer to the center of a test cartridge prepared with antibody as described above. Allow the buffer to absorb completely through the membrane.

2. Apply 30 µl of particulate free sample (1 ng/ml – 10 µg/ml of antigen) to the membrane (up to 200 µl of sample can be applied to the membrane before the system becomes saturated). Allow the sample to be completely absorbed.

3. Apply 3 drops of Universal Buffer to the center of the test cartridge to wash away any unbound sample.

4. Add 100-200 µl of your gold nanoparticles detection antibody conjugate prepared according to the procedure above to the test cartridge and allow the conjugate to absorb completely through the membrane.

5. (Optional) Add 3 drops of Universal Buffer to reduce any background and to increase the visibility of the result.

6. A red dot indicates that your target was present, the absence of a dot means that the target was not present, or was present at too low concentration to be detected, see figure 1.

7. The result can now be photographed or scanned.