

**miriad**<sup>™</sup>

**RVF Toolkit**



REF



Cat. No. 815311005930

**Store at 2-30°C**

For Research Use Only.

Not for use in diagnostic procedures.

Miriad RVF Toolkit contains all of the materials needed for creating a rapid analytical tool for the detection of antibodies or antigens using the patented MedMira Rapid Vertical Flow Technology<sup>™</sup>.

To detect antibodies using this toolkit the selected antigen(s) can be spotted onto the nitrocellulose membrane encapsulated within the specially designed plastic test cartridge. When the sample is applied any antibodies against the immobilized antigens in the sample will form an antigen/antibody immuno-complex which is detected through the use of the included InstantGold<sup>™</sup> Cap (See Table 1 for the description of the binding affinities of the InstantGold Cap towards various classes of antibody).

The kit is also equipped to detect specific antigen molecules. In this case a pair of antibodies, one for capturing the antigen and the other for gold conjugation, will be required. The capturing antibodies, monoclonal or polyclonal, can be spotted either individually or as a mixture onto the nitrocellulose membrane of the specially designed plastic test cartridge. The second antibodies used for the detection of the immuno-complex can be conjugated directly using the included Bare Gold. The presence of target antigen in the sample will form the antibody/antigen immuno-complex which is detected through the use of this second antibody-gold conjugate. A detailed conjugation procedure is provided.

In a fully optimized system, the limit of detection of this technology has been shown to be similar to colorimetric ELISA. However iterative optimization may be required to achieve this level of performance.

Each Miriad RVF Toolkit comes with:

- 50 test cartridges
- 50 InstantGold Caps
- 2 bottles of Universal Buffer (30 mL)
- 1 bottle of Bare Gold (5 mL, dark reddish solution)
- 50 disposable pipettes
- 1 Product Guide



**medmira**



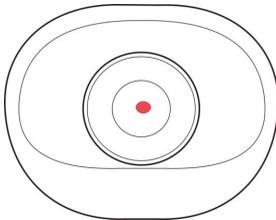
## Immobilization Procedure for Antibody Detection

1. Dilute the desired capture antigen to a working concentration using a spotting buffer (PBS, pH 7-8). Typical protein concentration range is in the order of 1 to 2 mg/mL.
2. Pipette the desired amount of the diluted capture antigen, typically between 0.5 and 1.0  $\mu\text{L}$ , 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 antigen 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 periods of up to 12 months, depending upon the nature of the antigen applied.

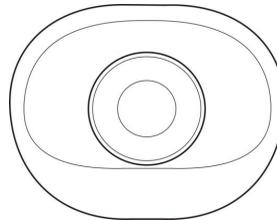
## Testing Procedure for Antibody Detection

1. Apply 3 drops of Universal Buffer to the center of the test cartridge and allow the buffer to absorb completely.
2. Apply 30  $\mu\text{L}$  of particulate free sample\* to the membrane (up to 200  $\mu\text{L}$  of sample can be applied to the membrane before the system becomes saturated). Allow the sample to be completely absorbed.
3. Place the InstantGold Cap onto the test cartridge. Dispense 12 drops of Universal Buffer into the InstantGold Cap and allow the solution to be completely absorbed.
4. When the buffer has been absorbed, remove the cap and read the results; it is optional to add 3 drops of Universal Buffer to reduce any background color and to increase the visibility of the result.
5. A red dot means that your target was present, the absence of a dot means that the target was not present, or was present at too low a concentration to be detected.
6. The results can be photographed or scanned.

**\*Only particulate free sample can be applied directly here, please consult with our Technical Support team for further details.**



Target Present



Target Not Present or  
Concentration Undetectable

## Antibody Gold Conjugation Procedure

1. Place the 5mL of dark reddish, Bare Gold solution supplied in the kit into a clean vial, and add 100  $\mu$ L of the selected antibody for conjugation at 1.0 mg/mL at the appropriate pH as described in Table 2.
2. Stopper the vial and place on a rocker set to a slow speed for approximately 1 hour at room temperature.
3. Add 100  $\mu$ L of 10% BSA at pH 8.2 to quench the reaction.
4. Store the conjugate in a refrigerator at 2-8°C until required. Do not store below 0°C. If the Bare Gold becomes colorless, contact MedMira Technical Support at support@medmira.com or T. 902 450 1588.

## Immobilization Procedure for Antigen Detection

1. Dilute the desired capture antibody to a working concentration using a spotting buffer (PBS, pH 8-9). 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  $\mu$ L, 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 be dried 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 to the membrane using a dryer set at low/medium heat for approximately 5 minutes.
4. The cartridges can then be stored at room temperature in a dry container with desiccants for periods of up to 12 months, depending upon the nature of the antibody applied.

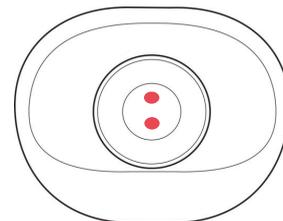
## Testing Procedure for Antigen Detection

1. Apply 3 drops of Universal Buffer to the center of the test cartridge and allow the buffer to absorb completely.
2. Apply 30  $\mu$ L of particulate free sample\* to the membrane (up to 200  $\mu$ L of sample can be applied to the membrane depending upon the amount required to obtain the desired sensitivity). Allow the sample to be completely absorbed.
3. Pipette 150  $\mu$ L of the prepared liquid antibody gold conjugate and allow the solution to be completely absorbed.
4. When the solution has been absorbed, read the results; it is optional to add 3 drops of Universal Buffer to reduce any background color to increase the visibility of the result.
5. A red dot means that your target was present, the absence of a dot means that the target was not present, or was present at too low a concentration to be detected.
6. The results can be photographed or scanned.

**\*Only particulate free sample can be applied directly here, please consult with our Technical Support team for further details.**

## Multiplexing Assays

It is very easy to perform tests for multiple analytes of the same nature (either all antigens or all antibodies) on the same cartridge by spotting down different proteins on the membrane surface. Care should be taken to avoid the spots running into one another during spotting as that could confuse reading of results. To obtain higher numbers of spots on the membranes, the volume spotted onto the membrane needs to be reduced.



Targets Present

## Technical support

MedMira offers full technical support for users of this product. Contact the Technical Support team at support@medmira.com, 1-877 MEDMIRA (toll free in N. America), or 902 450 1588, 8am to 4pm EST.

## TAG Conjugation Service

MedMira offers a conjugation service for users of the Miriad RVF Toolkit. MedMira TAG Conjugation Service has been designed to provide a rapid and cost effective supply for the small volumes of conjugate required for development and research use. For additional information contact Technical Support.

Table 1: Binding observed between antibody types and Protein A/L InstantGold Cap

Species	Antibody Type	Signal Strength
Human	IgG1, IgG2, IgG4	Strong
	IgG3, IgA, IgD, IgM, fab	Strong if certain kappa light chains are present
Mouse	IgG2a, IgG2b, IgG3	Strong
	IgG1, IgM	Strong if certain kappa light chains are present
Rat	IgG1; IgG2a, IgG2b, IgG2c	Strong
Rabbit/Dog/cat/guinea Pig	IgG	Strong
Cow/goat/sheep/horse/chicken	IgG	Weak

Table 2: Suggestions for pH Conjugation

Species	pH of Buffer
Mouse IgG (Polyclonal)	8.2
Mouse IgG (Monoclonal)	7.5
Rat IgG	8.5
Sheep IgG	9.2
Rabbit IgG	9.2
Chicken IgY	8.2

### Product Warranty

MedMira Laboratories Inc. guarantees the quality of this product if stored and used as instructed. Any component of the test found to be defective shall be replaced free of charge upon return of the defective product. MedMira Laboratories Inc. disclaims any implied warranty of merchantability or fitness for a particular purpose, and in no event shall MedMira Laboratories Inc. be liable for consequent damage.

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