Plasmodium vivax Microarray Kit

Part#: 24-MA-006K (40 array package)
Part#: 24-MA-007K (80 array package)
Part#: 24-MA-008K (160 array package)
Part#: 24-MA-009K (400 array package)

Intended use

This kit is for research use only. Kits are available in 40, 80, 160 and 400 arrays packages that are sufficient for 40, 80, 160 and 400 serum screening assays respectively.

Summary

ADi *Plasmodium vivax Malaria* Protein Microarray Chips are a highly multiplexed tool for measuring serum reactive antibodies to this pathogen. Each microarray chip contains up to 800 reactive proteins selected after extensive serological evaluation of a large proteome array [1]. The proteins on these microarrays were expressed by *in vitro* transcription/translation using an *E. coli* cell free expression system and printed onto nitrocellulose pads; each slide contains 8 separate pads. These *Plasmodium vivax* microarrays have been validated by probing serum collections from Malaria disease patients and healthy controls collected worldwide.

Materials supplied

Component	24-MA-006K Quantity	24-MA-007K Quantity	24-MA-008K Quantity	24-MA-009K Quantity
Plasmodium vivax 8-pad Protein Microarray Chip	5 slides	10 slides	20 slides	50 slides
Blocking Buffer	1x100 mL	1x100 mL	2x100mL	4x100mL
20X Wash Buffer	1x 15 mL	1x 15 mL	1x 30 mL	2x 30 mL
20X TBS	1x 7.5 mL	1x 7.5 mL	1x 15 mL	2x 15 mL
Secondary: biotin conjugated goat anti-human IgG Fcγ	1x 30 uL	1x 40 uL	1x 60 uL	1x 120 uL
Tertiary: streptavidin conjugated fluor	1x 70 uL	1x 120 uL	1x 220 uL	1x 520 uL
E.coli lysate (ECL)	1 tubes	2 tubes	4 tubes	10 tubes
Microarray Frame (holds 3 slides)	1	1	1	1
Microarray chambers	3	3	3	3
Microarray chamber clips	6	6	6	6

Note: Microarray frames, chambers and clips are reusable. Additional frames, chambers and clips can be purchased from GraceBio (cat# 246870, 246878 and 204838 respectively) or other vendors who can provide equivalent assembly.

Additional materials needed

1. Ultrapure water
2. Orbital shaker or rocker platform
3. Table top centrifuge
4. Slide scanner with a Cy5 compatible laser.
5. Pipettors and tips
6. Disposable polypropylene or polystyrene tubes for sample preparation
7. Disposable dispensing reservoirs
8. Containers for wash buffer dilution
9. Timer
10. Aspirator: can be assembled from a vacuum, Erlenmeyer flask, tubing and pipette tips.

Storage and handling

1. The product is shipped on cold pack or wet ice. Store all components at 4°C (2-8°C) except secondary and tertiary tubes which should be store at -20°C immediately upon receipt.
2. The kit is stable until the expiration date indicated on the label when stored as specified.
3. The microarray slides are provided in a sealed pouch with desiccant. Once opened, any unused portion should be reclosed with desiccant in the pouch and returned to 4°C storage immediately.

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4. Nitrocellulose pads should not be in direct contact with any solid object, e.g. a pipette tip, as they are susceptible to scratching.
5. Allow reagents to warm up to room temperature before use.
6. Do not use any reagent beyond the expiration date.
7. The diluted 1X Wash Buffer is stable the day of the preparation only.
8. Avoid exposure of fluorophore to light, air, or extreme temperatures.

Precautions

1. Read the manual before starting work.
2. Human serum is potentially pathogenic and should be handled in a class II biological containment hood. Wear appropriate PPE (disposable gloves, lab coat) when handling serum. Avoid aerosols and remove sharps from the work area. All liquid waste containing human serum should be inactivated with 10% (final concentration) chlorine bleach or equivalent.
3. All kit reagents should be warmed up to room temperature before use and handled according to good laboratory practice. Wear appropriate PPE (disposable gloves, lab coat). Use a dedicated workbench. No eating, drinking, applying cosmetics, smoking etc in the area. Follow laboratory guidelines for liquid and solid waste disposal or consult your biological safety officer.

Specimen collection and preparation

1. Collect serum or plasma specimens. Serum should be separated from red blood cells as soon as possible.
2. If storing specimens, avoid repeated freezing and thawing.

Reagent preparation

1. Reconstitute each lyophilized *E.coli* lysate (ECL) tube with 1.2 mL blocking buffer. Surplus lysate can be stored at -20°C.
2. Dilute wash buffer and TBS to 1X concentration. Diluted buffers are only stable the day of preparation. Be sure to only dilute the amount of buffer needed.
3. Prepare the stock solution of secondary antibody and tertiary detection fluor per manufacturer's instructions.

Procedure

DAY 1:

1. If frozen, thaw serum samples on ice.
2. Assemble the microarray frame, chamber and slide. Cover with a lid. The assembly illustration is shown as follows:

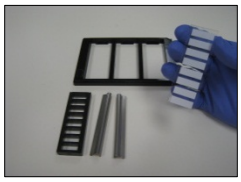
Figure 1

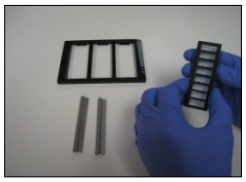
Figure 2

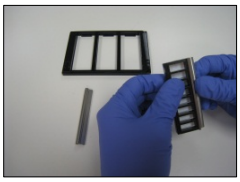
Figure 3

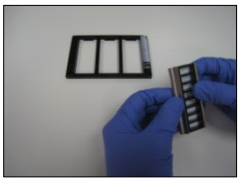
Figure 4

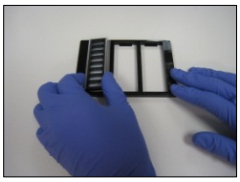
Figure 5

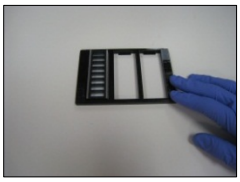
Figure 6

Figure 1. Frame, Chamber, clips and 8-pad slide before assembly
Figure 2. Align the 8-pad slide and the chamber
Figure 3. Snap one clip over one edge of the 8-pad slide/chamber
Figure 4. Snap another clip over the other edge of the 8-pad slide/chamber
Figure 5. Place the completed 8-pad slide/chamber/clip onto the frame
Figure 6. Finished assembly (up to 3 slides can be assembled into the frame)

3. Block the microarrays with 200 µl Blocking Buffer per well for 30-60 minutes.
4. Make 10% ECL in Blocking Buffer for pre-incubation of serum samples. Now referred to as pre-incubation solution.
5. In a Biosafety Cabinet, dilute serum 1 to 100 in pre-incubation solution and incubate for at least 30 minutes (200 µl/well needed for a 8-pad).
6. In a Biosafety Cabinet, remove blocking buffer and add diluted serum samples. Put the frames into a humidified airtight container. Incubate overnight at 4 degrees with gentle agitation.

DAY 2:

7. Wash with 200 µl 1X wash buffer per well 3 times for 5 minutes each.
8. Make 1% ECL in Blocking Buffer for dilution of secondary antibody (Biotin conjugated goat anti-human IgG Fcγ) and tertiary detection fluor (Streptavidin conjugated fluor). Now referred to as blocking solution.
9. Dilute secondary antibody 1 to 1,000 in blocking solution (200 µl/well needed for a 8-pad).
10. Remove wash buffer and add 200 µl diluted secondary antibody per well.

11. Incubate for 1 hour at room temperature.
12. Wash with 200 µl 1X wash buffer per well 3 times for 5 minutes each.
13. Dilute tertiary detection fluor 1 to 200 in blocking solution (200 µl/well needed for a 8-pad). Protect from light until ready to use.
14. Remove wash buffer and add 200 µl diluted tertiary detection fluor per well.
15. Incubate for 1 hour at room temperature covered with aluminum foil (i.e. in the dark).
16. Wash with 200 µl 1X wash buffer per well 3 times for 5 minutes each.
17. Wash with 200 µl 1X TBS per well 3 times for 1 minute each.
18. Fill a 2L beaker with ultra pure H_2O.
19. Remove slide from Fast frame assembly and dip in ultra pure water. Place into a 50 ml conical with a Kimwipe at the bottom. Spin at room temperature for 5 minutes at 500X g to dry.
20. Scan slides, quantify and analyze data.
    *Note: All incubation and washes are done on a rocking platform. First washes after incubation should be more thorough, using an aspirator, but subsequent washes may discard solution by dumping as long as wash buffer can be added quickly to prevent drying of the pads.

Quality control
Each array contains positive and negative control spots.

Data Analysis
Customers may choose their desired methods and software to perform the data analysis.
If desired, data analysis service may be arranged with ADi upon request.

Alternative Detections
The Biotin-Streptavidin fluorophore detection system provided in this kit has demonstrated the best sensitivity throughout our extensive research and development. However, customers may choose alternative procedures to detect the reactive antibodies to suit their specific needs. These include alkaline phosphatase conjugated secondary antibody with NBT/BCIP substrate system and fluorophore conjugated secondary antibody system.

Customers may detect the reactive antibodies in serum obtained from other animal by substituting a secondary antibody against the subject animal. For instance, if the customer is interested in detecting antibody in bovine serum samples, biotin conjugated goat anti-bovine IgG Fcr (which can be purchased from many sources) would be substituted for the human secondary antibody contained in this kit.

Limitations
This product is for research use only.

Troubleshooting

Symptom	Cause	Remedy
Low, or no, signal	• Scanning power is too low • Detection antibody did not bind • Tertiary did not bind • Fluor conjugate concentration is too low	• Increase the PMT/laser power • Use more concentrated sample
Saturated spots	• PMT/laser power too high	• avoid saturated spots (cannot be quantified), reduce PMT/laser settings
High background	• PMT/laser power too high • Brightness/contrast in software needs adjusting	• reduce PMT/laser settings • reduce brightness, increase contrast • background cannot be judged visually: measure background with analysis software, if possible use “intensity” measurements
High background and low signals	• Serum concentration too low • Insufficient blocking	• Increase serum concentration • Block longer (>30 min. to

	• Non-specific binding of fluor • Pads dried out during probing	overnight) • Reduce fluor concentration • Reduce the time between removing and adding liquids
Hazy/cloudy background/swirls	• Insufficient washing • Pads dried out during probing • Did not dry appropriately	• Increase the number of wash steps (minimum 3). Increase the Tween20 concentration in the wash buffer (up to 2% from original concentration of 0.05%) • Use humid chamber to prevent drying out of pads. • Do not allow the slides to sit without wash
Spot appears darker than surroundings	• Antibodies do not bind spot, proteins on the spot block binding of serum.	• Change blocking or incubation buffers.
Membrane scratches or damage	• Surface of the pad was touched	• Do NOT touch the surface of the pad
Spots with smear/comet like	• Serum, secondary or tertiary moves during incubation or washes	• Make sure to take extra precaution during the first wash, preventing too much agitation.
Speckles	• Precipitate is buffers • Particles in solutions	• Check and use fresh precipitate/particle free buffers
Control spots light up but not others	• Serum samples was not added	• Make sure to add the serum sample to the primary solution before addition to the chip

References

1.