Version 07-07/24

User's Manual

Monkeypox Virus (MPXV) A35R Antigen ELISA Kit

REF **DEIA-NS2311-3**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Monkeypox Virus A35R Antigen ELISA Kit is used for research purpose only of Monkeypox infections. It can be used to confirm the presence of the virus in a blood sample and to track the progress of the infection over time.

Reagents And Materials Provided

1. Capture Plate precoated with recombinant Monkeypox virus A35R antibody. 1 plate (96 wells)
2. Standard : Lyophilized Monkeypox virus A35R Antigen, 24,000 pg/vial (2 vials)
3. Detection A: Biotin labeled Anti-Monkeypox virus (A35R) antibody (including preservative), 120 µL (1 vial)
4. Detection B: HRP Conjugated Streptavidin , 120 µL (1 vial)
5. Standard Diluent, 25 mL (1 vial)
6. Assay Diluent, 25 mL (1 vial)
7. 20× Wash Solution, 25 mL (1 vial)
8. Color Reagent: TMB Solution, 12 mL (1 vial)
9. Stop Solution, 6 mL (1 vial)
10. Plate Sealer, 4 pieces

Storage

Stable for 6 months from the date of manufacture, if Capture Plate, Standard, Detection A and B are kept at -20°C. The remaining reagents can be stored at 4°C.

Specimen Collection And Preparation

Note: The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

Serum preparation procedure: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma preparation procedure: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Take all reagents from cold storage (-20 or 4°C) and allow them to return to room temperature before use

(20 to 25°C). Store all reagents at the recommended storage temperature promptly after use.

2. Vortex all reagents before use.
3. 1× Wash Solution preparation procedure: dilute the 20× Wash Solution in deionized or distilled water in a 1:20 volume ratio. Store the solution at 2-8°C when not in use. Example: to prepare 500 mL of this solution dilute 25 mL of 20× Wash Solution in 475 mL deionized or distilled water.
4. Detection A working solution preparation procedure: dilute Detection A in Assay Diluent in a 1:100 volume ratio.
5. Detection B working solution preparation procedure: dilute Detection B in Assay Diluent in a 1:100 volume ratio.
6. Standard preparation procedure: reconstitute the Standard with 1mL of **Standard Diluent.** This reconstitution produces a stock solution of 24,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making

dilutions. The 7 standard points were 24,000 pg/mL, 12,000 pg/mL, 6,000 pg/mL, 3,000 pg/mL, 1,500 pg/mL, 750 pg/mL, 375 pg/mL respectively. The appropriate Standard Diluent serves as the zero standard (0 ng/mL)

7. Sample preparation procedure: dilute your Sample with the Standard Diluent in a 1:10 volume ratio to be in the standard curve (this may need to be optimized according to the concentration of your sample). Example: to prepare 100 µL dilute 10 µL of each sample in 90 µL Standard Diluent.

Assay Procedure

For more accurate results it is recommended to measure each sample and control in duplicate.

1. Prepare the number of strips according to the number of samples you will be measuring. Install the strips making sure they are tightly snapped to the plate frame (see detailed protocol bellow).

   STEP 1: Hold the plate containing the preinstalled strips

   STEP 2: Carefully turn it over

   STEP 3: Press opposite wells A and H from the same strip

   STEP 4: Remove the loosen strip from the frame
2. Keep the unused strips in the closed foil pouch (to prevent damage from moisture) and store them at -20 °C.
3. Add 100 µL of the 10-fold diluted Standard or Sample to each necessary well of the Capture Plate.
4. Cover the Plate with the Plate Sealer and incubate at 37 °C for 120 minutes.
5. Remove the Plate Sealer and wash the plate with 300 µL 1× Wash Solution using a squirt bottle, manifold dispenser, or autowasher. Remove any residual liquid by inverting the plate and placing it over a clean paper towel to absorb residual droplets.
6. Repeat step (5) 3 times.
7. Add 100 µL Detection A working solution to each well.
8. Cover the Capture Plate with the Plate Sealer and incubate at 37 °C for 60 minutes.
9. Remove the Plate Sealer and wash the plate with 300 µL 1× Wash Solution using a squirt bottle, manifold dispenser, or autowasher. Remove any residual liquid by inverting the plate and placing it over a clean paper towel to absorb residual droplets.
10. Repeat step (9) 3 times.

11. Add 100 µL Detection B working solution to each well.
12. Cover with a new adhesive strip and incubate at 37 °C for 30 minutes.
13. Repeat washing steps 9 to 10.
14. Add 100 µL of Color Reagent to each well and incubate the plate in the dark at 37°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
15. Add 50 µL of Stop Solution to each well to quench the reaction. The color in the wells should change from blue to yellow. If the color is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
16. Read the absorbance in a microplate reader at 450 nm within 10 minutes. If wavelength correction is available, set it to 630 nm or 620 nm. If not, subtract readings at 630 nm or 620 nm from the readings at 450 nm to correct optical imperfections in the plate.
17. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Monkeypox Virus A35R Antigen concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: Substrate reaction time is determined by the reaction temperature. The ideal reaction temperature is 37 °C. If the temperature is lower than 37 °C, extend the reaction time appropriately.

Precision

CV<15%

Detection Range

1.25 ng/mL-20 ng/mL

Sensitivity

1 ng/ml