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User's Manual

Monkey B Virus (BV) Antibody ELISA Kit

REF **DEIA-JY2439**

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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 Monkey B Virus (BV) Antibody ELISA Kit is designed to detect the presence of IgG antibodies against B Virus in monkey serum or plasma samples.

Principles of Testing

The kit is based on the principle of the indirect enzyme-linked immunosorbent assay (ELISA). The B Virus-related whole virus protein antigens are pre-coated on the microplate. After adding the test serum, antibodies against B Virus in the sample specifically bind to the coated antigens. Subsequently, a secondary antibody labeled with horseradish peroxidase is added to form an antigen-antibody-enzyme complex. A colorimetric substrate for the enzyme is then added to induce color development in the reaction. Finally, the results are scanned using an enzyme-linked immunosorbent assay reader to determine whether anti-B Virus antibodies are present in the tested sample, thus diagnosing whether the tested animal is infected with B Virus.

Reagents And Materials Provided

1. **Pre-coated Microplate:** 1 plate (12 wells × 8 strips). Once opened, store in a resealable bag with desiccant at 2-8°C and use within 4 weeks.
2. **20× Wash Buffer:** 50 ml × 1. Phosphate buffer. Dilute to 1× Wash Buffer before use and store at room temperature for no more than 2 weeks.
3. **Sample Dilutant:** 5g × 1. Prepare with wash buffer before use and store at 2-8°C for no more than 2 days.
4. **Enzyme-Labeled Antibody:** 150 µl × 1. White cap. Dilute 1:100 with wash buffer before use and prepare fresh for immediate use.
5. **Positive Control:** 1 ml × 1. Purple cap. Ready to use.
6. **Negative Control:** 1 ml × 1. Green cap. Ready to use.
7. **HRP Substrate (TMB):** 12 ml × 1. Store at 2-8°C, avoid direct light.
8. **Stop Solution:** 5 ml × 1. Contains 1.25% sodium fluoride. Avoid direct contact with eyes and skin.

Materials Required But Not Supplied

1. Ultra-pure deionized water or distilled water
2. V-shaped liquid trough (20-50 ml) and 96-well deep plates (1 ml)
3. Shaker, plate washer, microplate incubator, and timer
4. Microplate reader with 405 nm and 490 nm wavelength filters
5. 10 µl and 1 ml pipettes with corresponding tips
6. 20-300 µl adjustable multichannel pipette (8 or 12 channels) with corresponding tips

Storage

Sealed kits store at 2-8°C until expiry date.

Specimen Collection And Preparation

1. Collect approximately 1.5 ml of venous blood using a blood collection tube. Take care to avoid rapid aspiration or shaking to prevent hemolysis.
2. Allow non-anticoagulant blood collection tubes to sit at room temperature for 30 minutes to allow blood to clot before aspirating the serum. For anticoagulant tubes, centrifuge at 1000 × g (about 2000-3000 rpm) for 10-15 minutes.
3. Use a 1 ml pipette to transfer the serum or plasma into a 2 ml centrifuge tube for storage or into a cryovial for freezing. Avoid reusing pipette tips during aspiration to prevent cross-contamination.
4. Samples may be stored at 2-8°C for short-term use; if storage exceeds five days, they must be frozen at -20°C.

Reagent Preparation

1. Before testing, remove the kit from storage and allow it to equilibrate to room temperature for at least 30 minutes before use.
2. Shake the 50 ml 20× Wash Buffer and pour it into a 2000 ml reagent bottle. Add 950 ml distilled water to prepare the 1× wash buffer (pH 7.2–7.4). If not used within two weeks, the wash buffer can be diluted proportionally. If crystals form, dissolve the reagent completely at 37°C before preparation.
3. Add 5 g Sample Dilutant to 100 ml of wash buffer to prepare a 5% sample dilution solution. Alternatively, prepare the solution based on the sample volume, assuming 1 ml per sample. Store any opened powder reagents in a dry, sealed container, as they are sensitive to moisture and may deteriorate.

Assay Procedure

1. **Sample Preparation:** Arrange the serum samples in a test tube rack and use a 10 µl pipette to add 10 µl of each serum into a deep well plate. Then, add 490 µl of sample diluent into each well using a multi-channel pipette. Cover the plate and gently shake it on a shaker. Remember to change the pipette tip for each sample to avoid contamination.
2. **Adding Samples:** For each experiment, set up positive and negative controls in separate wells. Add 100 µl directly to the corresponding wells on the pre-coated microplate. Transfer 100 µl of the diluted samples from the deep well plate to the microplate using a multi-channel pipette. If the sample volume is low, a single channel pipette can be used, but keep the process brief. Cover the plate and incubate it at 37°C for 30 minutes.
3. **Washing:** After incubation, carefully remove the cover, place the microplate on a plate washer, and wash with a low flow rate washing solution 5 times, using 350 µl per well. Remove any remaining liquid from the wells.
4. **Enzyme-Labeled Antibody:** Calculate the required amount of Enzyme-Labeled Antibody based on 120 µl per 96-well plate. Use a sterile pipette tip to aspirate the appropriate amount of enzyme-labeled antibody and add to the wash buffer. Dilute at a ratio of 1:100, mix thoroughly, and transfer the solution into a V-shaped liquid trough. Using a pipette, add 100 µl of the diluted enzyme-labeled antibody to each well of the microplate. Apply a sealing film and incubate the plate at 37°C for 30 minutes in an incubator.

5. Repeat the washing step 3.
6. **Color Development:** Place an appropriate amount of color developing reagent in a clean V-shaped reservoir and let it equilibrate to room temperature. Add 100 µl of the reagent to each well and incubate at room temperature (20-25°C) for 30 minutes.
7. **Termination (Optional):** If immediate reading is not possible, add 25 µl of termination reagent to each well using a multi-channel pipette to stop the color development reaction.
8. **Reading:** After color development, use a dual-wavelength microplate reader for immediate reading. Alternatively, add the termination reagent for 2 minutes and complete the reading within 15 minutes. (Test wavelength 405 nm, Reference wavelength 490 nm)

Quality Control

The OD value of the negative control must be < 0.24, and the OD value of the positive control must be ≥ 0.60. If these standards are not met, the experiment should be repeated.

Interpretation Of Results

OD_{sample} ≥ 0.3 is considered positive;

OD_{sample} < 0.3 is considered negative.

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