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<th>AVIDIN-BIOTIN SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY (ABS-ELISA) FOR THE DETECTION OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) VIRUS ANTIGEN</th>
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# Instructions for use

## Notes

- The AVIDIN-BIOTIN SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY (ABS-ELISA) is used for the detection of Hemorrhagic Fever with Renal Syndrome (HFRS) virus antigen.
- The method involves the use of avidin and biotin for the amplification of the antigen signal.
- Ensure proper handling of all reagents and standards to avoid cross-contamination.

## Procedure

1. **Preparation of Standards**: Prepare a series of standards containing known concentrations of HFRS virus antigen. Dilute the antigen in a buffer suitable for the assay.
2. **Sample Preparation**: Prepare the samples according to the protocol provided by the manufacturer. This may involve cell culture supernatants, tissue homogenates, or serum samples.
3. **Incubation**: Incubate the samples with the primary antibody (anti-HFRS virus antibody) for a specified time at room temperature or at 37°C.
4. **Washing**: Wash the wells to remove unbound antibodies.
5. **Addition of Biotin-labeled Secondary Antibody**: Add the biotin-labeled secondary antibody and incubate for a specified time.
6. **Washing**: Wash the wells again to remove unbound antibodies.
7. **Addition of Avidin-BSA Conjugate**: Dilute the avidin-BSA conjugate in the wash buffer and add to the wells. Incubate for a specified time.
8. **Washing**: Wash the wells once more to remove excess conjugate.
9. **Addition of substrate**: Add the substrate to the wells and incubate for a specified time to develop the color.
10. **Stop Reaction**: Stop the reaction by adding the stop reagent (usually sulfuric acid).
11. **Measurement**: Measure the optical density (OD) of the sample using a microplate reader at a wavelength of 450 nm.

## Interpretation

- The OD values are directly proportional to the concentration of HFRS virus antigen in the sample.
- Compare the OD values of the samples with the standard curve to determine the concentration of antigen.
- Positive results are indicated by OD values above a predetermined threshold.
AVIDIN-BIOTIN SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY (ABS-ELISA) FOR THE DETECTION OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) VIRUS ANTIGEN

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Sandwich enzyme-linked immunosorbent assay (S-ELISA) using avidin-biotin (AB) was newly developed to detect hemorrhagic fever with renal syndrome (HFRS) virus antigen. Anti-HFRS virus antibody as a capturing antibody (Cap. Ab) was fixed on the wells of a microplate and reacted with antigen. Biotinylated antibody was added to the wells as a detector antibody (Det. Ab), followed by peroxidase-labeled avidin and azino-di-(3-ethylbenzthiazothiazolic sulfonic acid). HFRS antigen was detected in the suspension of the organs from infected animals.

The results were summarized as follows:
1) As Cap. Ab to detect HFRS antigen, monoclonal antibody was found to be more sensitive than polyclonal antibody. Prozone phenomenon was seen in the high concentration of Cap. Ab against a constant level of antigen dilution. Absorbance value was constantly high at the range of $10^{-1}$ to $10^{-4}$ of Cap. Ab dilution and declined at further dilutions.
2) Absorbance value decreased in accordance with antigen dilution against the constant concentration of Det. Ab, and a clear dose-response curve was obtained.
3) Sensitivity of ABS-ELISA was almost the same as that of indirect ABS-ELISA, which used the secondary antibody. However, the level of non-specific reaction of ABS-ELISA was clearly lower than that of indirect ABS-ELISA. Absorbance value in the antigen control of ABS-ELISA was less by about 0.1 than that of indirect ABS-ELISA.
4) Suckling rats of less than 24 hr of age were inoculated intraperitoneally with SR-11 strain. Detection of antigen from the suspension of their organs was attempted by ABS-ELISA at intervals. In the lung suspension, antigen was detected at 6 days after virus inoculation and antigen titers were 1:32 to 1:64. In the brain suspension, antigen titers were 1:2 to 1:4 at 12 days after inoculation, and 1:32 at 16 days after inoculation. Furthermore, even in the presence of IFA antibody, the antigen was preserved in the organs of these rats for a long time. Rise of serum antibody titer was observed in a mother rat which was kept with infected suckling rats.
5) Detection of antigen was attempted by ABS-ELISA from the lung suspension of Rattus norvegicus rats captured in an endemic focus of HFRS. Antigen was detected in 6 of 26 rats, and IFA antibody titers were negative (less than 1:32) in 4 of the 6 antigen positive rats.