Immune Technology Corp.

The Resource for Virology Research

Schmallenberg Virus Nucleoprotein (NP) ELISA Development Kit

Catalog Number: IT-E3Ag-NP (Schmallenberg Virus)

BACKGROUND:

Schmallenberg virus, member of the Simbu serogroup of Orthobunyaviruses, was first identified in Schmallenberg of North Rhine-Westphalia, Germany in 2011. Schmallenberg virus infection causes congenital malformations and stillbirths in cattle, sheep, goats, and possibly alpaca. Since then, Schmallenberg virus infection has been reported in Netherlands, Belgium, France, Luxembourg, Italy, Spain, United Kingdom, Switzerland, Ireland, Finland, Denmark, Sweden, Austria, Norway, Poland, Estonia, and Great Britain. Schmallenberg virus infection has the potential to impact domestic dairy industry.

PRODUCT DESCRIPTION:

Nucleoprotein (NP) of Schmallenberg Virus is the most abundant viral antigen present in the virion and in the infected cells. This ELISA Development Kit contains the key components required for the quantitative analysis of NP of Schmallenberg Virus in cell culture supernatants, serum, and other biological samples within the range of 0.0316 - 31.6ng/ml in a sandwich ELISA format. Therefore, the kit can be used as a tool for detection of Schmallenberg Virus infection in species where the testing samples are derived. The components supplied in this kit are sufficient to perform the assay in approximately 500 ELISA plate wells.

REAGENTS PROVIDED

Capture Antibody: 100µl of 1mg/ml anti-NP (Schmallenberg Virus) monoclonal antibody.

Standard Protein: 50µl of 50µg/ml recombinant NP (Schmallenberg Virus).

Detection Antibody: 50µl of biotinylated monoclonal antibody against NP (Schmallenberg Virus).

Streptavidin-HRP Conjugate: 25µl of HRP- conjugated streptavidin.

RECOMMENDED MATERIALS (not included in the kit)*

ELISA 96-well plates: Corning Prod # 3590 or equivalent plate

Block Buffer: 5% skim milk in PBS

Wash Buffer: 0.05% Tween-20 in PBS

Diluent: 0.05% Tween-20, 0.1% skim milk in PBS

Substrate: TMB Peroxidase Substrate

Stop Solution: 2N Sulfuric Acid

****Alternatively, purchase Immune Tech ELISA Plate/Buffer/Substrate Kit: Cat* #IT-200-002.**

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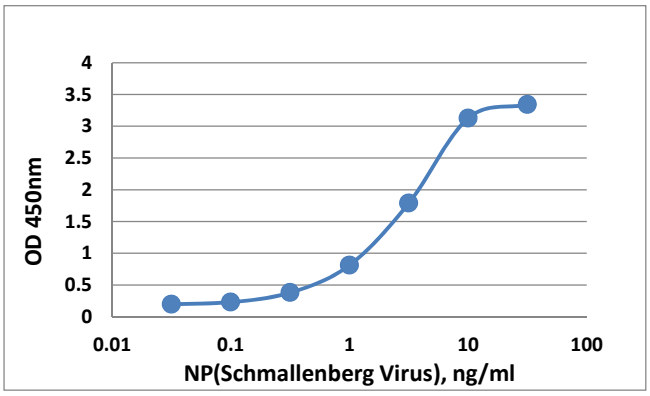
PLATE PREPARATION

1. **Plate coating:**
   a. For each 96-well plate, dilute 20µl of capture antibody with 10.5ml of PBS.
   b. Add 100µl to each well immediately.
   c. Seal the plate and incubate overnight at 4°C.
2. **Washing**
   a. Remove the coating reagent by aspirating or decanting.
   b. Invert the plate and blot on the paper towel.
3. **Blocking and Washing**
   a. Add 300µl of block buffer to each well.
   b. Incubate for at least 1 hour at room temperature.
   c. Aspirate the wells to remove liquid and wash the plate 4 times with 300µl of wash buffer per well.

ASSAY PROCEDURE

1. **Standard/Sample preparation:**
   a. Dilute standard with Diluent to the following eight concentrations
   --31.6ng/ml,
   --10ng/ml
   --3.16ng/ml
   --1ng/ml
   --0.316ng/ml
   --0.1ng/ml
   --0.0316ng/ml
   --0ng/ml
   b. Make a serial dilution of testing samples with Diluent
   c. Immediately, add 100µl of standard and sample to each well in triplicate.
   d. Incubate at 37°C for 1 hour.
2. **Detection:**
   a. Aspirate and wash plate 4 times.
   b. Dilute 10µl of detection antibody with 10.5ml of PBS.
   c. Add 100µl into each well.
   d. Incubate at 37°C for 1 hour.
3. **Streptavidin Peroxidase:**
   a. Aspirate and wash plate 4 times.
   b. Dilute 5µl of Streptavidin-HRP conjugate with 10.5ml of PBS.
   c. Add 100µl into each well.
   d. Incubate at 37°C for 45 minutes.
4. **Substrate/Stop:**
   a. Aspirate and wash plate 4 times.
   b. Add 100µl of TMB Peroxidase Substrate into each well.
   c. Incubate at 37°C for 25 minutes.
   d. Add 100µl of stop solution to each well.
5. **Read:** Determine the optical density of each well within 30 minutes, using a microplate reader set to 450nm.
6. **Analysis:**
   a. Average the triplicate readings from the standard, control, and sample
   b. Subtract the average zero standard optical density
   c. Generate a standard curve by applying the data using computer software capable of generating a four parameter logistic (4-PL) or other curve-fit.
   d. NP (Schmallenberg Virus) concentration in sample can be determined by regression analysis.
   e. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Figure 1. Standard curve with serial dilution of protein standard.



Reference
John R. Crowther. The ELISA Guidebook (Methods in Molecular Biology), Humana Press, 2000.