Jul 12, 2022

Neutralizing antibody detection

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dx.doi.org/10.17504/protocols.io.bdati2en

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ABSTRACT

Neutralizing antibodies of serum samples were detected by 90% plaque reduction neutralization test (PRNT90).

DOI

dx.doi.org/10.17504/protocols.io.bdati2en

PROTOCOL CITATION

Shihong Fu, Wenjing Liu 2022. Neutralizing antibody detection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bdati2en

CREATED

Mar 06, 2020

LAST MODIFIED

Jul 12, 2022

PROTOCOL INTEGER ID

33843

MATERIALS TEXT

serum samples ,Japanese encephalitis virus P3 isolate,West Nile virus,BHK-21 cell.

BEFORE STARTING

the serum samples should be inactived (56℃，30min,in water)before the test.

Neutralizing antibody detection

1 1. Diluted serum: dilute the serum sample in a 96-well plate. Take 24 µl of inactivated serum and mix it with 96 µl of MEM culture medium to complete the 5-fold dilution of serum,the Serum samples were serially diluted two-fold beginning at a 1:5 dilution and ending at 1: 160 .

2 2. Dilute virus: use MEM culture medium to dilute virus to 200 pfu/100 µl.

3 3. The diluted serum (from 1:5-1:160) was mixed with an equal volume of 200 pfu of JE virus (strain P3). At the same time, different dilutions of virus (100 PFU, 10 PFU, 1 PFU) were set as controls, and the diluted virus solution was mixed with serum samples and incubated at 37 ° C and 5% CO2 for 1 h.

4 4 Incubation of infected cells with the neutralized mixture: inoculate 6-well plates of BHK-21 cells grown to 90% confluence in monolayer, and incubate at 37°C, 5% CO2 for 1 h.

5 5 Overlay methylcellulose medium culture: supplement 4 ml of 1.2% methylcellulose-MEM medium containing 2% FBS, culture at 37°C and 5% CO2 for 3-5 days until obvious viral plaque is observed.

6 6. Stain and calculate the neutralization: remove the culture medium, stain with crystal violet for 1h, gently rinse with running water, dry and count the number of viral plaques, and calculate the neutralizing antibody of JE virus in the sample.

Citation: Shihong Fu, Wenjing Liu Neutralizing antibody detection https://dx.doi.org/10.17504/protocols.io.bdati2en