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Working

DENV Titration

Fabio Gomes[1]
[1]Johns Hopkins University

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Fabio Gomes

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Fixation of Cells

1 Prepare fixative - 80% methanol in water

2 Dump Methylcellulose overlay and blot on paper towels

3 Wash gently with 1X PBS. Incubate for 10 min. Blot and dry. 00:10:00

4 Add 0.5 mL fixative to each well. Allow plates to sit at room temperature for 10 min.

Note: Fixed cells can be stored at -70oC for future use. Leave methanol on cells when freezing.

Antibody

5 Wash plates with PBS. Incubate for 10 min. 00:10:00

If frozen plates are being used, incubate at 37oC for 30 min.

6 Prepare antibody dilutions in 5% skim milk, PBS [M]5 Mass/Volume Percent **Skim Milk**

4G2 1:2000

7 Add 200 µl to each well. Incubate at 37oC for 1 hour on a rocker. 01:00:00

37 °C

Minimize contact with each well by maintaining contact of the pipette tip with the wall of well.

8 Dump off primary antibody solution and tap plates on paper towels to remove excess solution

9 Wash with 1 mL 5% skim milk, PBS

10 Prepare antibody dilutions in 5% skim milk, PBS.

Anti-mouse-Per 1:2,000

11 Add 200 µl to each well. Incubate at 37oC for 1 hour on a rocker. 01:00:00 37 °C

Color Development

12 Dump off antibody solution. Tap on paper towels

13 Wash twice with PBS. Tap on paper towel

14 Add 160 µl of TrueBlue substrate per well

15 Place on rocker at RT until plaques develop (10 min or longer).

16 Dump off peroxidase substrate.

17 Count plates or incubate at 4oC for up to 7 days.