Datasheet & Protocol

FociSpot Path: Influenza B (NP)

4406-4AT-1

4406-4AT-10

This kit is intended for FociSpot immunostaining of the influenza B nucleoprotein (NP), which enables enumeration of influenza virus foci in Focus Forming Assays (FFA). For research use only. Not for use in diagnostic procedures.

Contents		size -1	size -10
Detection mAb	Anti-influenza B (NP) mAb (4D5), biotin 0.5 mg/ml	15 µl	150 µl
Enzyme conjugate	Streptavidin-ALP	15 µl	150 µl
Substrate	BCIP/NBT-plus	25 ml	120 ml
Plate	96-well tissue culture treated plate	1	10

To ensure total recovery of the stated quantity, vials and bottles have been overfilled.

Shipping and Storage

- Shipped at ambient temperature.
- Store reagents at 4-8 °C upon receipt. Plates should be kept at room temperature.
- The mAb is supplied in sterile filtered PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.
- The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

FociSpot

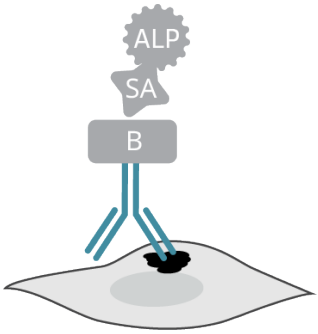
The reagents supplied in the kit enable the immunostaining and detection steps of FFA. The preceding steps, including cell culture and virus infection, should be set up in the supplied plate.

Specificity

The monoclonal antibody in this kit is specific for the influenza B nucleoprotein (NP). The assay has been validated using influenza infected MDCK cells.

Assay principle

In FociSpot, virus foci are detected by using mAbs specific to viral proteins. After fixation and permeabilization of infected cells in a 96-well plate, the virus-specific biotinylated detection mAb is added. The use of Streptavidin-ALP enzyme conjugate, followed by a precipitating substrate enables detection of foci. Foci are preferably counted with Mabtech IRIS 2, which offers high-throughput counting and easy data handling. Foci can also be counted manually using a microscope.



Precautions

- The protocol for virus infection of cells needs to be established by the user prior to starting the FociSpot assay.
- To prevent release of virus progeny and infection of surrounding cells, Oseltamivir acid (50 nM) should be added to the cell media.*
- Consult local regulations for handling of virus and cells, considering biological hazards and waste handling.
- Formaldehyde and methanol used for fixation and permeabilization are chemicals with hazard classifications. Consult safety data sheets from the manufacturer and follow local regulations.

Reference

*Guosong Wang, et al. (2021), *Establishment of a rapid ELISPOT assay for influenza virus titration and neutralizing antibody detection*. **Journal of Medical Virology.**

Protocol

1. Remove medium from the plate and fixate the cells by adding 200 µl per well of 4% formaldehyde diluted in PBS. Incubate for 15 minutes at room temperature.
2. Wash the plate 4 times with PBS, 200 µl per well.
3. Add 100 µl per well of ice-cold methanol (99.8%) to permeabilize the cells. Incubate for 10 minutes at room temperature.
4. Wash as above.
5. Add 200 µl per well of PBS with 1% BSA to block the plate. Incubate for 1 hour at room temperature.
6. Wash as above.
7. Add 100 µl per well of the detection mAb (4D5-biotin) diluted to 0.5 µg/ml in PBS with 0.1% BSA. Incubate for 1 hour at room temperature.
8. Wash as above.
9. Add 100 µl per well of Streptavidin-ALP diluted 1:1000 in PBS with 0.1% BSA. Incubate for 1 hour at room temperature.
10. Wash as above.
11. Filter the BCIP/NBT-plus substrate through a 0.45 µm filter and add 100 µl per well. Develop until distinct foci emerge (5-30 minutes). Stop color development by washing thoroughly in tap water.
12. Leave the plate to dry (plates should be completely dry before analysis). Store plates protected from light at room temperature and preferably analyze within 1 week of development.

MABTECH

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The products are for research use only.

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

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