Product No. B-3658
Monoclonal Anti-Blood Brain Barrier
Purified Mouse Immunoglobulin
Clone HT-7

Monoclonal Anti-Blood Brain Barrier (BBB) (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from Balb/c mice immunized with 7-day chick nasal half-retina, immunosuppressed with cyclophosphamide, as immunogen.[1] The product is purified by Protein A affinity chromatography. The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Monoclonal Anti-Blood Brain Barrier (clone HT-7), recognizes a highly glycosylated 74 kD protein expressed on the surface of chick embryonic blood cells. The antibody recognizes chicken embryonic blood cells and brain endothelial cells but not other endothelial cells. The BBB antigen, a specific endothelial cell marker, is expressed in the epithelial cells of kidney tubules, choroid plexus epithelium (site of the blood-cerebrospinal fluid barrier), retinal pigment layer, erythroblast and pinealocytes.[2, 3] BBB antigen belongs to the immunoglobulin superfamily, but its function has not yet been clearly defined.[4]

Immunoglobulin Concentration: 1.0 mg/ml

Working Dilution

A working dilution of at least 1:800 was determined by indirect immunohistology using 14-21 day old chick brain frozen sections.

Uses

Monoclonal Anti-Blood Brain Barrier may be used for:
1. Studies in the development and maturation of chicken brain cells.
2. Studies in the barrier and transport functions performed by specialized endothelial cells

Storage

Store at 2-8°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

1. Matthew, W., and Paterson, P., Cold Spring Harbor Symp. Quant. Biol., **48**, 625 (1983).
2. Risau, W., et.al., EMBO J., **5**, 3179 (1986).
3. Seulerger, H., et.al., EMBO J., **9**, 2151 (1990).
4. Ikeda, E., et al., Neurosci. Lett., **209**,1 (1996).

Procedure for Immunohistological Staining

Reagents and Materials Needed but Not Supplied

1. Dulbecco's Buffered Phosphate Saline (PBS), pH 7.5 (Sigma Product No. D-5652).
2. Blocking Buffer: PBS containing 3% normal goat serum (Sigma Product Nos. P-3813 and G-9023).
3. Negative Control: Mouse IgG1(Sigma Product No. M-9035 or M-9269).
4 Primary Antibody: Monoclonal Anti-Blood Brain Barrier (Sigma Product No. B-3658).
5. Secondary Antibody: FITC Conjugated Anti-Mouse IgG (Sigma Product No. F-9006).
6. 14-21 day old chick brain frozen sections on slides coated with 0.5% gelatin.
7. Humidity chambers (Sigma Product No. H-6644).
8. Fluorescence Microscope, 10X and 40X objectives, equipped with a 35 mm camera.

Procedure

Positive control cell lines should be included in the procedure whenever possible. Examples of positive controls may be chick epithelial cells of kidney tubules or retinal pigment layer.

1. Prepare sample slides.
2. Fix cells in methanol –70°C for 5 minutes.
3. Wash samples 3 times in PBS for 30 minutes at room temperature.
4. Block slides for 30 minutes with blocking buffer.
5. Add diluted primary antibody to slide. Incubate in dark humidity chambers for 1 hr.
6. Wash slides with a gentle stream of PBS for 30 seconds.
7. Add secondary antibody diluted appropriately. Incubate for 30 minutes.
8. Allow slides to air dry. Examine slides with a fluorescence microscope.

Quality Control

Running appropriate negative control cells is advisable. Negative control cells should always be included to establish a baseline.