Technical Data Sheet

Purified Mouse anti-Transportin

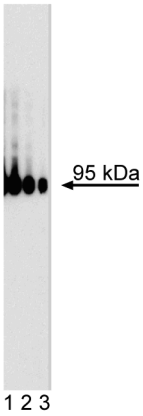
Product Information

Material Number:	**558660**
Alternate Name:	karyopherin β2, importin β2, M9 region interaction protein
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	36/Transportin
Immunogen:	Human N-terminal Transportin
Isotype:	Mouse IgG1, κ
Reactivity:	Routinely tested: Human Confirmed during development: Rat
Target MW:	95 - 101 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

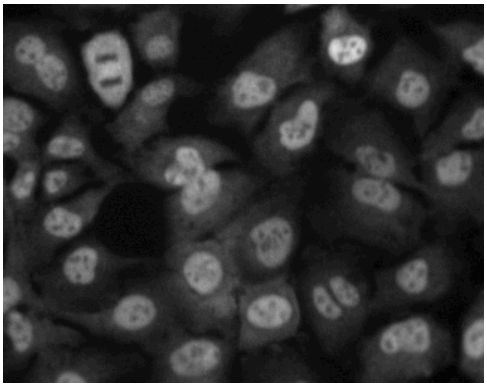
Description

Transport of macromolecules into and out of the nucleus occurs via interaction of several cytosolic and nuclear pore proteins. The nuclear pore complex (NPC), anchored in the nuclear envelope, mediates active transport of proteins and RNA into and out of the nucleus.

Transportin is 890 amino acids in length and a member of the karyopherin β (also known as importin β) superfamily of soluble transport proteins. It specifically interacts with a ~38-amino acid basic domain (M9) of its cargoes (proteins and ribonucleoproteins), the polypeptides of the NPC, and RanGTP (the GTP-bound form of the Ras family protein Ran), which modulates cargo binding. The gradient of RanGTP across the nuclear envelope regulates the transport process.



Western blot analysis of transportin in human T leukemia. *Jurkat cell lysate (Cat. No. 611451) was probed with Mouse anti-transportin monoclonal antibody at concentrations of 0. 125 (lane 1), 0.0625 (lane 2), and 0.03125 µg/ml (lane 3). Transportin is identified as a band of 95 - 101 kDa.*



Immunofluorescent staining of HeLa (ATCC CCL-2) cells. *Cells were seeded in a 96-well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the Mouse anti-Transportin monoclonal antibody. The second-step reagent was Alexa Fluor® 555 goat anti-mouse Ig (Invitrogen). Images were captured on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Triton is a trademark of the Dow Chemical Company.

References

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Pollard VW, Michael WM, Nakielny S, Siomi MC, Wang F, Dreyfuss G. A novel receptor-mediated nuclear protein import pathway. *Cell.* 1996; 86:985-994. (Biology)

Poon IKH, Jans DA. Regulation of nuclear transport: central role in development and transformation?. *Traffic.* 2005; 6:173-186. (Biology)

Ribbeck K, Kutay U, Paraskeva E, Görlich D. The translocation of transportin-cargo complexes through nuclear pores is independent of both Ran and energy. *Curr Biol.* 1999; 9:47-50. (Biology)