

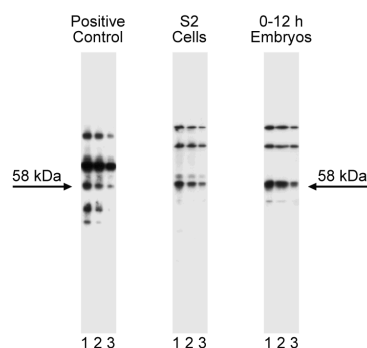
Technical Data Sheet

Purified Mouse Anti-Karyopherin α **Product Information**

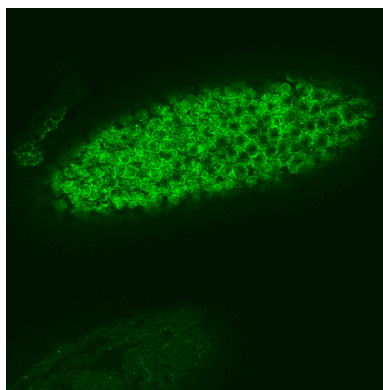
Material Number:	612654
Alternate Name:	Rch-1
Size:	50 μ g
Concentration:	250 μ g/ml
Clone:	2/Karyopherin α
Immunogen:	Human Rch-1 aa. 254-497
Isotype:	Mouse IgG1
Reactivity:	Drosophila
Target MW:	58 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and $\leq 0.09\%$ sodium azide.

Description

The two step process of importing proteins into the nucleus involves the binding and interaction of several cytosolic and nuclear pore proteins. Proteins to be translocated into the nucleus contain a nuclear localization sequence (NLS) which is recognized and bound by carrier proteins in the cytosol. Heterodimers belonging to a highly conserved family of proteins called karyopherins are required for successful nuclear localization of cytosolic proteins. The α -subunits appear to function in the binding of NLS (both simple and bitartite NLS motifs), but both α - and β -subunits are required for successful docking to the nuclear envelope. ATP is required for complete translocation of proteins into the nucleus. Karyopherin $\alpha 2$ was first identified as Rch-1, an NLS receptor which interacts with the RAG-1 recombination-activating protein in developing B and T cells. Rch-1 has been reported to be 44% identical to karyopherin $\alpha 1$ (hSRP-1 /NPI-1).



Western blot analysis of Karyopherin α . Lysates from HeLa cells (5 μ g/lane), S2 cells (7.5 μ g/lane), and 0-12 hours Drosophila embryos (7.5 μ g/lane) were probed with the mouse anti-karyopherin antibody at concentrations of 1 μ g/ml (lane 1), 0.5 μ g/ml (lane 2), and 0.25 μ g/ml (lane 3). The mouse anti-karyopherin α antibody detects a band of 58 kDa in all lysates.



Immunofluorescence staining of 0-12 hour embryos. Formalin-fixed embryos kept in ethanol at -20°C , were re-hydrated and incubated with the mouse anti-karyopherin antibody at 1 μ g/ml in TBST. After extensive washes to remove unbound antibody, signal was visualized by confocal microscopy with an Alexa Fluor® 488 anti-mouse secondary antibody (1:300 dilution). (A) Ventral view of a cellularizing embryo. Staining is perinuclear. (B) High magnification view of figure A; staining is in the nuclear envelope and dots in the nucleus.

Preparation and Storage

Store undiluted at -20°C .

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

Application Notes**Application**

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development

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Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

The *Drosophila melanogaster* gene karyopherin $\alpha 3$ (abbreviated as Kap- $\alpha 3$) encodes a protein carrier involved in protein-nucleus import which is a component of the importin complex. Its amino acid sequence contains an importin b binding domain. Similar sequences have been identified in *C. elegans*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, and *Saccharomyces cerevisiae*. It has been mapped cytologically to 85D24. [FBgn0027338] (The FlyBase Consortium (<http://flybase.org/>))

>gb|AAF54408.1|(AE003683) karyopherin-alpha3 gene product [*Drosophila melanogaster*] Length = 514
Score = 262 bits (669), Expect = 1e-70
Identities = 127/244 (52%), Positives = 179/244 (73%), Gaps = 2/244 (0%)

Query: 1 PTLVRLHHDDPEVLADTCWAISYLTGPNERIGMVVKTGVVPQLVKLLGASELPIVTPA 60
P L L+HH D +L DT WAISYLTDG N++I MV+++GVVP+L+ LLG SE+ + T A
Sbjct: 240 PALNVLIHHTDNTILVDTVWAISYLTGQNDQIQMVIESGVVPKLIPLLGNSEVKVQTAA 299

Query: 61 LRAIGNIVTGTDEQTQVVIDAGALAVFPSLLTNPKTNIQEATWTMSNITAGRQDQIQV 120
LRA+GNIVTG+DEQTQVV++ AL+ FP LL++PK I+KEA W +SNITAG Q Q+Q V
Sbjct: 300 LRAVGNIVTGSDEQTQVVLNYDALSYFPGLLSHPKEKIRKEAVWFLSNITAGNQSQVQAV 359

Query: 121 VNHGLVPFLVSVLSKADFKTQKEAVWAVTNYTSGGTVEQIVYLVHCGIIEPLMNLLTAKD 180
+N GL+P ++ LSK +F+TQKEA WA++N T G EQ+ L+ G+I P +LL+ +D
Sbjct: 360 INVGLLPKIIENLSKGEFQTQKEAAWAISNLTISGNREQVFTLIKEGVIPPFCDLLSCQD 419

Query: 181 TKIILVILDAISNIFQAAEKLGETEKL SIMIEECGLDKIEALQNHENESVYKASLSLIE 240
T++I V+LD ++N+ + A+ E ++IEEC GL KIE LQ+HEN +YK + +I+
Sbjct: 420 TQVINVL DGLNMLKVAD - -SHVEAVANCIEECGLAKIERLQSHENVEIYKLAYEIID 477

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Moroianu J, Blobel G, Radu A. Previously identified protein of uncertain function is karyopherin alpha and together with karyopherin beta docks import substrate at nuclear pore complexes. *Proc Natl Acad Sci U S A*. 1995; 92(6):2008-2011. (Biology)

Moroianu J, Hijikata M, Blobel G, Radu A. Mammalian karyopherin alpha 1 beta and alpha 2 beta heterodimers: alpha 1 or alpha 2 subunit binds nuclear localization signal and beta subunit interacts with peptide repeat-containing nucleoporins. *Proc Natl Acad Sci U S A*. 1995; 92(14):6532-6536. (Biology)

Weis K, Mattaj JW, Lamond AI. Identification of hSRP1 alpha as a functional receptor for nuclear localization sequences. *Science*. 1995; 268(5213):1049-1053. (Biology)