Technical Data Sheet

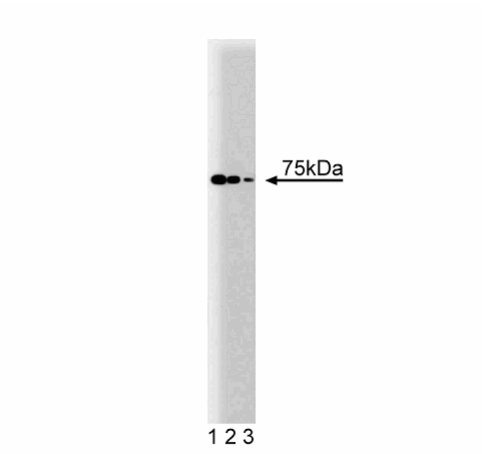
Purified Mouse Anti-Human LEDGF

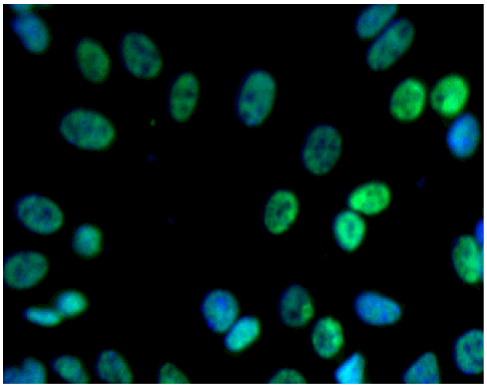
Product Information

Material Number:	**611714**
Alternate Name:	Lens Epithelium-Derived Growth Factor
Size:	50 µg
Concentration:	250 µg/ml
Clone:	26/LEDGF
Immunogen:	Human LEDGF aa. 85-188
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	75 kDa and/or 52 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

p52/p75 proteins were first identified through their interaction with transcriptional factors and splicing factors. p75 is encoded by exons 1-15 and contains 530 amino acids, while the alternatively spliced p52 is encoded by exons 1-9 and contains 333 amino acids. Both p52 and p75 have strong interactions with the VP16 activation domain and with various components of the general transcriptional machinery. p52 is the more active transcription activator and has also been shown to recruit the pre-mRNA splicing factor ASF/SF to sites of active transcription. Interestingly, p52/p75 has also been identified as Lens Epithelium-Derived Growth Factor (LEDGF). In serum free media, LEDGF stimulates the growth and survival of a variety of cells, such as lens epithelial cells, retinal photoreceptor cells, COS7 cells, skin fibroblasts, and keratinocytes. In retinal photoreceptor cells, LEDGF is found in the nucleus where it promotes resistance to serum starvation and thermal stress. Thus, the LEDGF gene encodes splice variants that may regulate gene transcription and mRNA splicing of a variety of proteins that are essential for cell growth and survival.





Western blot analysis of LEDGF on a HeLa cell lysate (Human cervical epitheloid carcinoma; ATCC CCL-2.2) (left). *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the mouse anti-human LEDGF antibody.*

Immunofluorescent staining of SK-N-SH cells (Human neuroblastoma; ATCC HTB-11) (right). *Cells were seeded in collagen coated 384-well imaging microplates (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) and the mouse anti-human LEDGF antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen) (pseudo-colored green) and counter-stained with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 855 or 435 Bioimager system using a 20x objective and merged using BD Attovision™ software. This antibody also stained SH-SY5Y (Human neuroblastoma; ATCC CRL-2266), C6 (Rat glioma; ATCC CCL-107), U-87 MG (Human glioblastoma cells; ATCC HTB-14) and U-373 cells (Human glioblastoma cells; ATCC HTB-17; discontinued, investigators may refer to: http://www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx) using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development

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

Bioimaging:
Methanol Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

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)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

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

References

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Ge H, Si Y, Wolffe AP. A novel transcriptional coactivator, p52, functionally interacts with the essential splicing factor ASF/SF2. *Mol Cell.* 1998; 2(6):751-759. (Biology)
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Singh DP, Kimura A, Chylack LT Jr, Shinohara T. Lens epithelium-derived growth factor (LEDGF/p75) and p52 are derived from a single gene by alternative splicing. *Gene.* 2000; 242(1-2):265-273.(Biology)