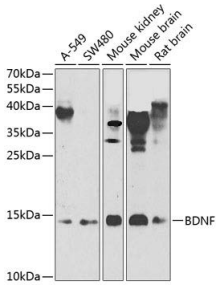
Product Data Sheet: BDNF (Mature Form)

Cat. No:	AB-83595
Conjugate:	Unconjugated
Size:	100 ug
Clone:	POLY
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	Recombinant protein of human BDNF
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot: 1:1000 Immunohistochemistry (paraffin-embedded tissues): 1:50-100 Immunofluorescence
Molecular Weight:	14 kDa (mature BDNF) ~ 28-37 kDa (precursor BDNF)
Purification:	Aff. Pur.
Background:	This gene encodes a member of the nerve growth factor family of proteins. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature protein. Binding of this protein to its cognate receptor promotes neuronal survival in the adult brain. Expression of this gene is reduced in Alzheimer's, Parkinson's, and Huntington's disease patients. This gene may play a role in the regulation of the stress response and in the biology of mood disorders.
Form:	Liquid
Buffer:	PBS with 0.02% sodium azide, 50% glycerol, pH7.3.
Storage:	Store at -20° C for 12 months. Avoid freeze and Thaw cycles.



Western blot analysis of extracts of various cell lines, using BDNF antibody at 1:1000 dilution.
Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) at 1:10000 dilution.
Lysates/proteins: 25ug per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL West Pico Kit.
Exposure time: 90s.

Wester blot using SH-SY5Y differetiated with ATRA/TPA and treated by 6OHDA and antagonist/agonist of specific receptors for 24 hours, exposition 3 minutes. (Lane 1: Ctr, Lane 2: 6OHDA, Lane 3: 6OHDA+ antagonist, Lane 4: 6OHDA+agonist, Lane 5: 6OHDA+antagonist+agonist).
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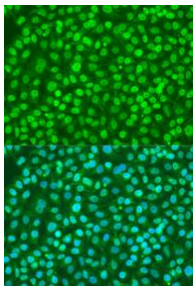
Western Blotting Protocol of mBDNF:
1) Prepare 13% acrylamide gel.
2) Load 30µg sample per lane.
3) Start Electrophoretic run in a running buffer (25mM Tris, 190mM Glycine, 0,1% SDS) and keep 50 Volts continuously until the end of stacking gel.
4) After the stacking gel increase up to 100-150 Volts .
5) After the electrophoresis run put the



Immunofluorescence analysis of U2OS cells using BDNF antibody at dilution of 1:100. Blue: DAPI for nuclear staining.

gel in double distilled H2O .
6) Prepare the sandwich for the blotting phase.
7) Il blotting is made both by the Wet and Semi-dry systems, using the same Transfer Buffer (25mM Tris, 190mM Glycine, 20% Methanol)
8) Transfer conditions in Wet for 1mm gel: 1 hour at 400mA constant in ice. For 1,5mm gel: 1 hour and half at 400mA constant.
9) Transfer conditions in Semi-dry: for 1mm gel 1 hour at max 25V and 1A, for 1,5mm gel the same conditions were used, and the transfer lasts 1 hour and 15 minutes.Western Blot Image given by courtesy :
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10) After the transfer lay the membranes in H2O double distilled and cut each membrane according to the markers needed for the analysis, usually for mBDNF which has a molecular weight of 15kDa the cut is made to permit the binding of the primary antibody only to the mature form, excluding the other form detected to higher molecular weights.
11) Blocking with (non fat dry milk) al 5% in TBS-T (0,1% di Tween 20) for 1 hour at RT.
12) Incubation: overnight at 4°C with primary anti-BDNF at a dilution of: 1:1000.
13) The day after recover the primary antibody and keep at -20°C start washing the membrane, usually 3 washes 5-10 minutes with TBS-T (0,1% di Tween 20) at RT.
14) Incubate the secondary antibody (dilution from: 1:10,000 to :1:20,000, in TBS-T) for 1 hour at RT.
15) 4 Washes for 5-10 minutes with TBS-T at RT.
16) Acquire the chemiluminescence signal in of the bands, the signal is obtained through the use of ECL kit and the acquisition is performed through Chemidoc UVITEC.