

Cat. No: AB-82598
Conjugate: Unconjugated
Size: 100 ug
Clone: POLY
Concentration: 1mg/ml
Host: Rb
Isotype: IgG

Immunogen: E.coli-derived human BDNF recombinant protein (Position: H129-R247). Human BDNF shares 100% amino acid (aa) sequence identity with both mouse and rat BDNF.

Reactivity: Hu,Ms,Rt

Applications:

- Western Blot : 1:500 – 1:1000
- IHC(paraffin embedded): 1:500 – 1:1000
- IF (Immunofluorescence):1:50

Molecular Weight: 37 kDa , 25 kDa

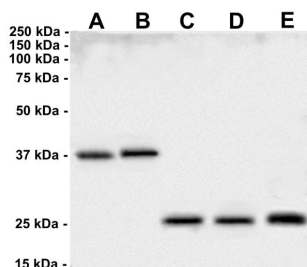
Purification: Aff. Pur.

Background: During development, promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous systems. Participates in axonal growth, pathfinding and in the modulation of dendritic growth and morphology. Major regulator of synaptic transmission and plasticity at adult synapses in many regions of the CNS. The versatility of BDNF is emphasized by its contribution to a range of adaptive neuronal responses including long-term potentiation (LTP), long-term depression (LTD), certain forms of short-term synaptic plasticity, as well as homeostatic regulation of intrinsic neuronal excitability.

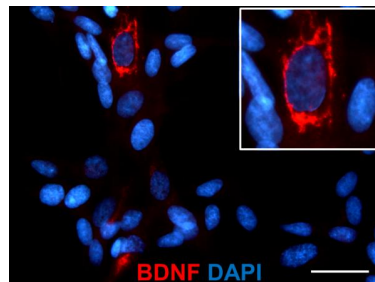
Form: Liquid

Buffer: Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Storage: 4° C for 2 months for longer terms at -20°C. Avoid Freeze and thaw cycles.

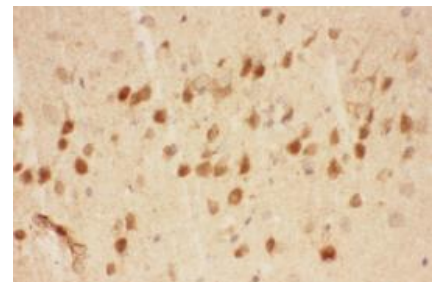


Prof.Rosa Di Liddo , Dr. Thomas Bertalot,
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 Department of Pharmaceutical and
 Pharmacological Sciences
 See western blot protocol on Western
 Blotting AB-82598
 (Protocol of Prof.Rosa Di



Immunofluorescence AB-82598 (Protocol
 of Prof.Rosa Di Liddo,Dr. Thomas
 Bertalot,Dr. Sandra Schrenk)
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Procedures



IHC(P): Mouse Brain Tissue
 By Heat: Boiling the paraffin sections in
 10mM citrate buffer, pH6.0, for 20mins is
 required for the staining of
 formalin/paraffin sections

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Sample preparation

Whole protein lysate was obtained using RIPA lysis buffer supplemented with protease inhibitors. All procedures were performed on ice and homogenization of samples was executed using a 22G needle syringe.

1. SDS-PAGE Electrophoresis: Sample loaded: 10µg – Poly-Acrylamide gel at 12%

2. Transfer: 0.45µm PVDF Membrane (Transfer for 80 min at 4°C on ice) -140 Volts and 14 mA

3. Blocking: – Blocking solution: 5% no-fat milk with TBS -Time: 2h

4. Incubation of Primary antibody:
Dilution of primary antibody: 1:1000 [1µg/mL] -Dilute buffer: 1% no-fat milk with TBS - Time and temperature: 4°C overnight in constant slow shaking

5. Washing: Wash with TBS-T (Tween-20, 0.25%) for three times (15 min, 5 min, 5 min)

6. Incubation of Secondary antibody:
Dilution of secondary antibody: HRP-Goat anti-rabbit IgG (1:2500)

7. Dilute buffer: 1% no-fat milk with TBS -Time and temperature: RT, 90min on shaker

8. Washing: Wash with TBS-T (Tween-20, 0.25%) for four times (20 min, 5 min, 5 min, 5 min)

9. ECL chemiluminescent method:

1. Cell culture

ENSc were seeded on glass coverslips and maintained in standard medium for 7 days

2. Fixation

ENSc were fixed using a paraformaldehyde solution kit for 20 min at 4°C

3. Permeabilization

Samples were permeabilized using a 0.5% Triton X-100 solution prepared in PBS 1X for 15 min at RT

4. Blocking

Blocking solution: 5% BSA in PBS 1X for 45 min at RT

5. Incubation of Primary antibody

Dilution of primary antibody: 1:50 [20µg/mL]

Dilute buffer: 0.5% BSA in PBS 1X
Time and temperature: 4°C overnight

6. Washing

Wash with PBS 1X for three times

7. Incubation of Secondary antibody

Dilution of secondary antibody: PE-Goat anti-rabbit IgG (1:100) [10µg/mL]- Dilute buffer: 0.5% BSA in PBS 1X
Time and temperature: RT, 60min

8. Washing

Wash with PBS 1X for three times

9. Mounting

Mount the sample with Fluoromont G with DAPI