Technical Data Sheet

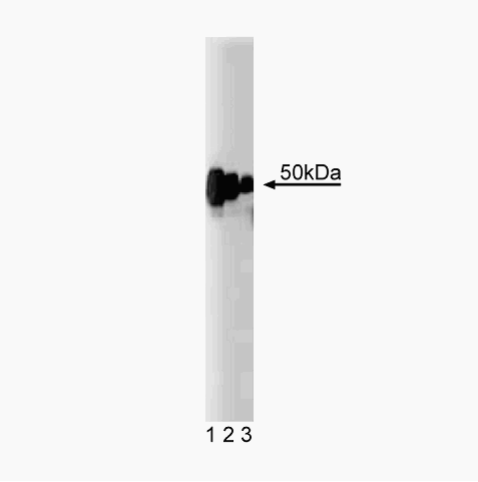
Purified Mouse Anti-Carboxypeptidase E

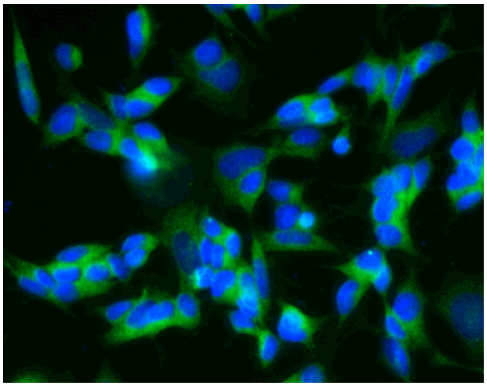
Product Information

Material Number:	**610759**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	35/Carboxypeptidase E
Immunogen:	Human Carboxypeptidase E aa. 49-200
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat Tested in Development: Human, Mouse
Target MW:	50 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Carboxy**p**eptidase **E** (CPE), also known as carboxypeptidase H and enkephalin convertase, is found as both a membrane-bound and a soluble glycoprotein in neuroendocrine tissues and adrenal-gland chromaffin granules. The C-terminus forms an amphiphilic α-helix, suggesting that this region is responsible for the membrane-bound form. Evidence suggests the active form of CPE is located in the secretory vesicles. CPE appears to have several functions. It is an exopeptidase that cleaves neuropeptides with C-terminal basic amino acids, producing an active form of the peptide. It has also been proposed that membrane-bound CPE is a sorting receptor for regulated secretory pathway (RSP) proteins in the TGN pituitary Golgi and secretory granule membranes. RSP proteins primarily consist of hormones and neuropeptides. Mice that carry a mutation in the CPE gene *Cpe[fat]* display endocrine disorders such as obesity, infertility, and hyperproinsulinemia. Furthermore, the same endocrine disorders are observed in *Cpe[fat]* mice where the CPE gene has been effaced by antisense RNA.





Western blot analysis of Carboxypeptidase E on rat brain lysate (left panel). *Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of Carboxypeptidase E,*

Immunofluorescent staining of SH-SY5Y cells (right panel). *Cells were seeded in a collagen coated 384 well imaging plate (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton X100 fix/perm protocol (see Recommended Assay Procedure) and the anti-Carboxypeptidase E antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). The image was taken on a Pathway 855 or 435 imager using a 20x objective. This antibody also stained SK-N-SH and C6 cells using both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

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

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Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	Not Recommended

Recommended Assay Procedure:

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
611463	Rat Cerebrum Lysate	500 µg	(none)

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

Cool DR, Normant E, Shen F. Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in Cpe(fat) mice. *Cell.* 1997; 88(1):73-83.(Biology)

Manser E, Fernandez D, Loo L. Human carboxypeptidase E. Isolation and characterization of the cDNA, sequence conservation, expression and processing in vitro. *Biochem J.* 1990; 267(2):517-525.(Biology)

Shen FS, Loh YP. Intracellular misrouting and abnormal secretion of adrenocorticotropin and growth hormone in cpefat mice associated with a carboxypeptidase E mutation. *Proc Natl Acad Sci U S A.* 1997; 94(10):5314-5319.(Biology)

Varlamov O, Fricker LD. The C-terminal region of carboxypeptidase E involved in membrane binding is distinct from the region involved with intracellular routing. *J Biol Chem.* 1996; 271(11):6077-6083.(Biology)