COSMO BIO CO., LTD.
Inspiration for Life Science

Catalog No. LSL-LB-3117

Anti (bov,MAP) Prion protein A

1. Description

Host animal	Rabbit
Source (Volume)	Whole serum (100ul)
Titer	According to the ELISA assay, results are positive for dilutions up to 50,000 fold against linear synthetic peptides.
Source of antigen	bovine Multiple Antigen Peptide *
Cross reactivity	Cross react with bovine, sheep, human, rat and mouse.*
Characteristic	Appears as positive band by Western blotting. Detects a nerve cell by Immunohistochemistry
Standard dilution	More than 1:100 dilution using Immunofluoresence method. More than 1:500 dilution using a Western blotting.

* Cross reaction will differ between species. The above dilution is only a recommendation and the optimum concentration may differ for each case.
The MAP sequence 17 aa including *a*structure" -YYQ-" is consistent with mouse/rat 11/17 aa ,human 13/17 aa and sheep 17/17 aa.

2. Storage

Store below -20℃ (below -70℃ for prolonged storage).
After thawing, store in small aliquots in sealable vials and store below -70℃. To prevent degradation from repeated thawing, store the antiserum between 0 to 4℃ after second thawing.

3. Stability

Stable for three years at -70℃.
This product does not contain preservatives such as NaN_3.

For research use only; not for use as a diagnostic.

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カタログＮｏ．ＬＢ－３１１７

Anti (bov. MAP) Prion protein A

Ⅰ．内容　　Ｌｏｔ　Ｎｏ．７１２０２２

免疫動物	ウサギ
性状・包装ｻｲｽﾞ	全血清・１００μℓ
力価	ELISA で50,000倍希釈まで陽性（合成直鎖ペプチドに対して）
抗原由来	ウシ Multiple Antigen Peptide ＊
種間交差	ウシ、ヒツジ、ヒト、ラット、マウスと交差＊
特徴	ウシ及びラット・脳粗抽出物を用いた Western Blottingで prion protein の分子量に相当する位置に陽性バンドを確認。免疫組織染色で神経系細胞が良く染色される。
標準希釈率	蛍光抗体法で１：１００以上 (1/50～1/500) Western Blottingに関しては１：５００以上

（　）内は推薦希釈率

＊種によって交差性が異なると思われます、希釈率等充分に検討下さい。但し、ＭＡＰに合成したウシ prion proteinのＣ端付近α構造を含む部位のーＹＹＱーを含むアミノ酸配列（１７残基）はマウス・ラットの同一部位のアミノ酸配列と１１／１７、ヒトと１３／１７、ヒツジと１７／１７一致している。

Ⅱ．保存上の注意

－２０℃以下（長期間の場合は－７０℃以下）で凍結して下さい。
解凍後は密栓のできる小型容器に研究の規模に応じて少量づつ分注し、－７０℃以下で保存して下さい。
凍結融解の繰り返しによる力価の低下を避ける為、再解凍後の抗血清は０～４℃に保ち操作・保存して下さい。

Ⅲ．安定性

－７０℃で３年間安定。
但し、ＮａＮ₃等の防腐剤は入っていません。

製造元　　総発売元

LB-3117
LB-3227

RAPID COMMUNICATION

Immunohistochemical detection of cellular prion protein (PrPc) in the rat central nervous system

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ABSTRACT

A simple conventional method of immunohistochemistry (i.e. fixing the frozen sections in cold methanol) was used to determine the immunolocalization of cellular prion protein (PrPc), with good results. In the rat cerebrum, the cytoplasm of neural cells in the cortex and corpus stratum, pia mater, membrane limitans gliae superficialis, choroid plexus and blood vessel wall were immunostained. The formation of network structures of immunostained neural and/or glial fibers in the cerebral cortex was also observed. These immunostained network structures of neural and/or glial fibers were also observed in cultured neural cells. The results suggest that fixation of frozen sections and cultured cells with cold methanol is a useful method for detecting the immunolocalization of PrPc and that PrPc exists in the various components of the central nervous system of the rat.

KEYWORDS: cellular prion protein, glial cell, immunohistochemistry, neuron.

There have been many reports on the localization of scrapie prion protein (PrPsc) and the associated neurodegenerative changes, and an immunohistochemical method for detecting PrPsc has been established (Van Everbroeck *et al.* 1999; Debeer *et al.* 2001; Ikeda *et al.* 2002; Kovacs *et al.* 2002). However, because of the importance of PrPsc as the major transmissible agent of a group of neurodegenerative diseases called the transmissible spongiform encephalopathies (TSEs), efforts have mainly been directed toward understanding the infection, and the physiological roles of cellular prion protein (PrPc) have largely been ignored (Martins *et al.* 2002). An immunostaining method for PrPc has not been established, and results regarding its immunohistochemical localization in nervous tissue have been contradictory (Laine *et al.* 2001).

Some groups have reported the requirement of perfusion fixation, microwave treatment and/or pre-embedding staining for retrieval of the antigenicity of PrPc or for immunodetection of PrPc in brain tissue (Verghese-Nikolakaki *et al.* 1999; Laine *et al.* 2001). We tried a simple method: fixing frozen sections or cultured cells in cold methanol.

MATERIALS AND METHODS

Samples

Experiments were performed in compliance with the guidelines of the Graduate School of Agriculture Hokkaido University. Whole brains were excised under deep ether anesthesia from three 5-week-old-male Wistar strain rats and from ICR strain mouse embryos on day 15 of gestation.

The excised rat cerebra were embedded in an OCT compound (Tissue Tek; SAKURA, Tokyo, Japan), and frozen frontal sections (10 μm in thickness) were cut in a cryostat (CM3000; Leica, Germany) and mounted on glass slides (Lee *et al.* 2000).

After removing the connective tissue, the rinsed whole brains of the mouse embryos were dissected into small pieces and treated enzymatically (Suzuki

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Received 25 June 2002; accepted for publication 26 July 2002.

1999). The neurospheres were prepared according to the method of Reynolds *et al.* (1992) and plated on laminin-coated culture slides (Becton Dickinson Labware, Lincoln Park, NJ, USA). The cells were cultured in Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham (Sigma, St Louis, MO, USA) supplemented with 25 μg/mL insulin (Sigma), 100 μg/mL transferrin (Nacalai Tesque Inc., Kyoto, Japan), 20 nmol/L progesterone (Sigma) and 60 μmol/L putrescine (Nacalai) for 3 days at 37°C in a humidified atmosphere of 5% CO_2 in air (Reynolds & Weiss 1992; Reynolds *et al.* 1992; Kubo *et al.* 2000).

Antibodies

For raising anti-PrPc antibodies, two oligopeptides corresponding to the bovine PrPc sequence at amino acids 148–164 (A) and 219–235 (B) were synthesized and coupled to multiantigenic peptides (MAP; Asahi Techno Glass, Tokyo, Japan). The two kinds of MAP were mixed with Freund's adjuvant and injected into rabbits (Nakamura *et al.* 2000). In western blotting, anti-PrPc A and B antisera stained the same bands originating from the PrPc of bovine and rat brains, but did not cross-react with other protein bands of lysis buffered (150 mmol/L NaCl, 2 mmol/L EDTA, 0.5% triton X-100, 0.5% Na-deoxycholate, 50 mmol/L Tris, pH 7.5) extracts of brains (Groschup & Pfaff 1993; Ma & Lindquist 2001). These positively stained bands were lost when the extracts were treated with proteinase k (Prusiner 1998; Gu *et al.* 2002; Ikeda *et al.* 2002).

The secondary antibody was a fluorescein isothiocyanate (FITC)-conjugated goat IgG to rabbit IgG (ICN Pharmaceuticals Inc., Cappel Products, West Chester, PA, USA).

Immunofluorescence

Brain sections and cultured cells that had been rinsed with phosphate-buffered saline (PBS) were fixed with 100% cold methanol at −20°C for 20 min (Ma & Lindquist 2001) and rehydrated with PBS. To prevent non-specific antibody binding, the sections and cells were incubated for 30 min at 37°C in a blocking

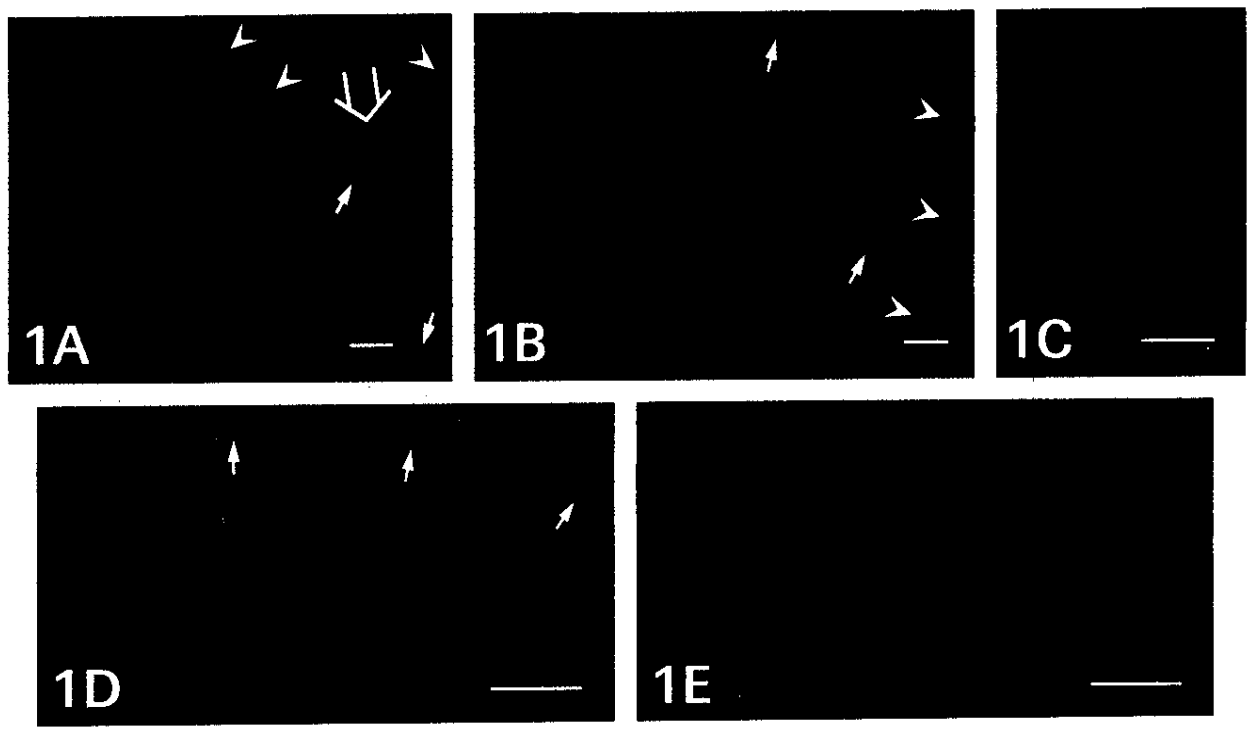

Fig. 1 Immunofluorescences of cellular prion protein (PrPc) in frontal sections of the rat cerebrum. In the cerebral cortex near the longitudinal fissure (large arrow in A), pia mater (arrowheads), vascular periphery (small arrows) and neural cells of the external granular layer and the layer of pyramidal cells are stained. Near the lateral ventricle (B), choroid plexus (arrowheads), small blood vessel wall (small arrows) and neural cells of the corpus striatum are stained. Neural cells (C) throughout the cytoplasm, especially the nuclear periphery, are stained. (D, E) Two kinds of fibrous staining pattern in the molecular layer of the inferior cortex, and membrane limitans gliae superficialis or tangential fibers (arrows) are also stained. Scale bars, 20 μm.

solution containing 5% normal goat serum (Sigma), 3% casein (Nacalai) and 1% bovine serum albumin (Sigma) in T-PBS (0.05% Tween-20 containing PBS). The sections and cells were incubated sequentially with anti-PrPc A antiserum diluted 1 : 100 in blocking solution for 90 min at 37°C and washed with T-PBS. After reincubation with the secondary antibody diluted 1 : 150 in blocking solution for 30 min at 37°C, the sections and cells were washed extensively with T-PBS and coverslipped with Perma Fluor (Shandon/Lipshow, Pittsburgh, PA, USA), and then observed under an epifluorescence microscope.

For controls, sections were stained after preabsorption of the primary antibody with the lysis buffer extracts of bovine brain or were stained without the primary antibody.

RESULTS AND DISCUSSION

Abundant PrPc immunoreactivity was observed in the rat cerebral frozen sections fixed in cold methanol (Fig. 1). However, there was not a clear positive immunoreaction of PrPc in the formalin-fixed frozen sections (data not shown), suggesting that some treatment to retrieve the antigenicity of PrPc is required for its immunodetection in formalin-fixed brain tissue (Verghese-Nikolakaki *et al.* 1999; Laine *et al.* 2001). In the cerebral cortex near the longitudinal fissure, neural cells of the external granular layer and the layer of pyramidal cells were stained, and pia mater, especially in the vascular periphery, was stained intensely (Fig. 1A). Intense staining was also observed in the ependyma of the lateral ventricle, and the choroid plexus, small blood vessels and neural cells of the corpus striatum showed positive immunoreactions (Fig. 1B). It was clear that the cytoplasm of almost all neural cells was stained, and the perinucleus was shown to be stained intensely in higher magnification images of the corpus striatum (Fig. 1C). On the other hand, in the inferior cortex, two kinds of staining pattern in the high magnification images of the molecular layer were found. As can be seen in Fig. 1D, the membrane limitans gliae superficialis or tangential and branched perpendicular fibers of Retzius and/or Cajal's cells were stained, and they formed a large meshed network structure. As can be seen in Fig. 1E, astrocytes and gliofibrils were stained, and they formed a small, but widespread, meshed network.

We found that PrPc existed in many different components of the central nervous system *in vivo*. Next, we observed the expression of PrPc in neural cells *in vitro*.

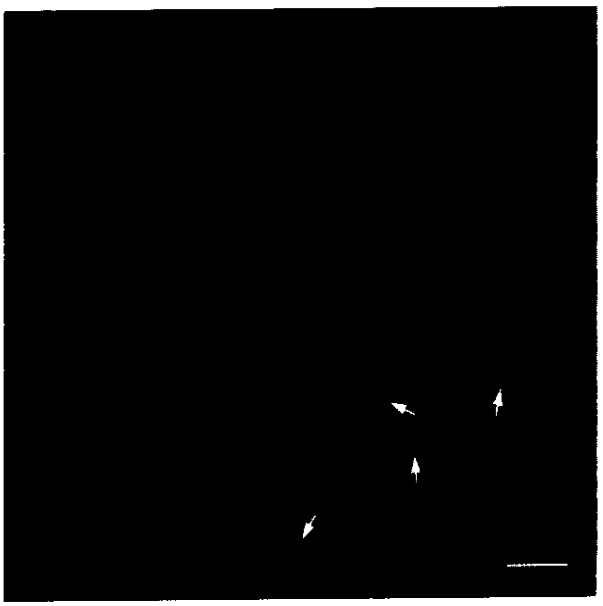

Fig. 2 Immunofluorescence of cellular prion protein (PrPc) in cultured mouse nerve cells. The cytoplasm around the nuclei (arrows) and the cell processes of outgrown neuronal and/or glial cells from the neurosphere are stained. Scale bar, 20 μm.

It is well known that the neurosphere is composed of neural stem cells, which differentiate into neural and glial cells when they attach to and outgrow on laminin (Lein & Higgins 1989; Reynolds & Weiss 1992; Reynolds *et al.* 1992; Suzuki 1999). Abundant PrPc immunoreactivity is also shown in Fig. 2. Stained neural and/or glial cell processes formed various network structures, some of which resembled that shown in Fig. 1D and others which resembled that shown in Fig. 1E. Additionally, the staining appearance of cell bodies resembled that shown in Fig. 1C. These findings regarding the immunostained nervous cells *in vitro* suggest that the abundant PrPc immunoreactivity in rat frozen sections reflects the usual expression of PrPc in the cerebrum *in vivo*. In other words, a simple conventional method of histochemistry (i.e. fixing a frozen section in cold methanol) is one of the most useful methods for detecting the immunolocalization of PrPc in tissues.

Although we have not described PrPc localization in detail or over a large area, we consider that PrPc mapping in various tissues will be an essential preliminary step for determining the physiological role of PrPc and the relationships between PrPc and PrPsc.

ACKNOWLEDGMENT

We are grateful to the staff of LSL Co. (Tokyo) for their advice on the preparation of the anti-PrPc antibodies.

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