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## ENZYME IMMUNOASSAY KIT FOR THE DETERMINATION OF PrPc

catalogue # A05201

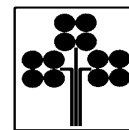
96 wells

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U.S. patent # 50 47 330  
European patent # 89 139 552

**THE ENZYME IMMUNOASSAY KIT FOR  
THE DETERMINATION OF PrPc HAS  
BEEN DEVELOPED AND VALIDATED  
BY SPI-BIO.**

*For research laboratory use only.  
Not for human diagnostic use.*



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## EIA KIT FOR THE DETERMINATION OF PrPc

96 wells  
Storage: -20°C  
Expiry date: stated on the package

This kit contains:

- ☞ A covered 96 wells plate, pre-coated with anti-Prion Protein mouse monoclonal antibody, ready to use
- ☞ One vial of anti-Prion Protein tracer, lyophilised
- ☞ Two vials of Prion Protein positive control, lyophilised (dry cow milk)
- ☞ One vial of EIA buffer, lyophilised
- ☞ One vial of concentrated wash buffer, liquid
- ☞ One vial of tween 20, liquid
- ☞ Two vials of Ellman's reagent, lyophilised
- ☞ One instruction booklet
- ☞ One template sheet
- ☞ Two well cover sheets

Each kit contains sufficient reagents for 96 wells.

### PRECAUTIONS FOR USE

**Users are recommended to read all instructions for use before starting work.**

Each time a new pipet tip is used, aspirate a sample or reagent and dispense it back into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.

Not for diagnostic use.

Do not pipet liquids by mouth.

Do not use kit components beyond the expiration date.

Do not eat, drink or smoke in area in which kit reagents are handled.

Avoid splashing.

The total amount of reagents contains less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

### PRINCIPLE OF THE ASSAY

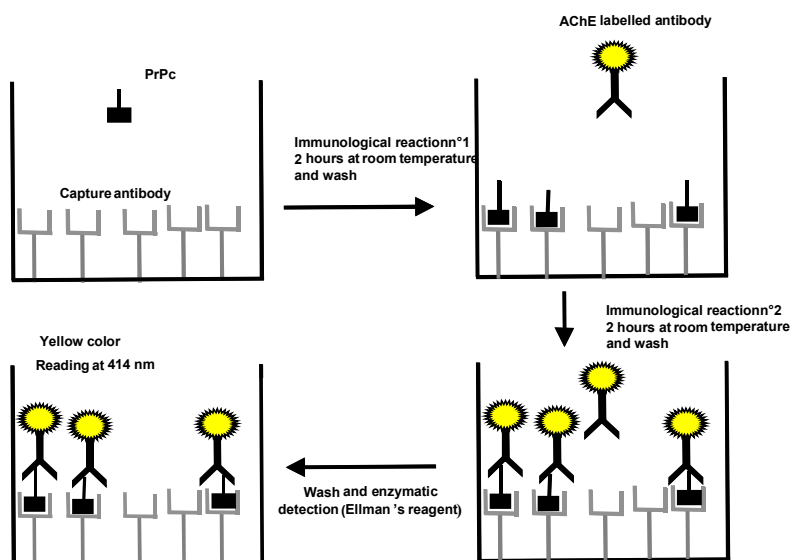
This assay has been validated for the detection of native PrPc in brain extracts. It can also be used to detect PrPc extracted from other tissues. The immunometric assay also detects denaturated PrP, as well as recombinant PrP. Although the antibodies used in this kit have been raised against hamster PrP, they cross-react with PrP from most of mammalian species including mouse, human, sheep and cattle.

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate are coated with a monoclonal antibody specific to the Prion Protein which recognises the protein sequence DYEDRYREN within amino acids 144-153 (human numbering). The acetylcholinesterase (AChE) - Fab' conjugate which recognises the octo-repeat region located in the N-terminal part of PrPc is also added to the wells.

These two antibodies were raised against a preparation of denaturated SAFs (Scrapie Associated Fibrils) from infected hamster brain.

The sandwich is immobilised on the plate so the excess reagents may be washed away. The detection of the PrPc is then determined by measuring the enzymatic activity of the immobilized AChE using the Ellman's Reagent. The AChE tracer acts on the Ellman's Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the PrPc present in the well during the immunological incubation. This proportionality is specific for each specie. The principle of the assay is summarised below:



## MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

### FOR SAMPLE PREPARATION

- ☞ Tris-HCl, NaCl, EDTA, IGEPAL CA-630, Deoxycholic Acid, Sodium Azide, Urea.
- ☞ Grinding tube
- ☞ Tissue homogenizer : Ribolyser should be preferentially used but other types of tissue homogeniser such as potter can be used as well.
- ☞ Distilled or deionized water
- ☞ Eppendorf tubes 1.5 ml

### FOR THE ASSAY

- ☞ Precision micropipettes ( 20 to 1000 µl)
- ☞ Spectrophotometer Plate Reader (405 or 414 nm filter)
- ☞ Microplate washer (or wash-bottles)
- ☞ Microplate shaker
- ☞ Distilled or deionized water
- ☞ Polypropylene tubes



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## SAMPLE PREPARATION

### GENERAL PRECAUTIONS

- ☞ This assay has been validated only for the determination of soluble native PrPc as found in a brain extracts but it also work with PrPc extracted from other tissues. The sandwich immunoassay also detects denaturated PrP as well as recombinant PrP.
- ☞ All samples must be free of organic solvents prior to assay.
- ☞ Samples should be assayed immediately after collection or should be stored at -80°C.

### SAMPLE PREPARATION

Since PrP is a membranous protein, it has to be extracted from tissues using a mixture of detergents before being assayed (see procedure below). Homogenisation of the tissue is obtained using grinding tubes containing ceramic beads and submitted to a violent agitation in a Ribolyser machine. After homogenisation tissue fragment are separated from the soluble extract by centrifugation. The assay can only be performed on this extract which must be diluted at least 1/10 in EIA buffer in order to remove interference due to the presence of detergents.

### EXTRACTION BUFFER PREPARATION

- 10 mM Tris-HCl pH 7.4
- 0.1 M NaCl
- 0.01M EDTA
- 0.5 % IGEPAL CA-630 (SIGMA ref I-3021)
- 1% Deoxycholic Acid (SIGMA ref D-6750)
- Sodium Azide 0.01 %

### EXTRACTION PROTOCOL

- ☞ Extracts have to be frozen at -20°C or, preferably -80°C and can be stored undiluted for several months. They can only be submitted to 3 freezing / thawing cycles.
- ☞ Weight 175 mg +/- 20 mg of tissue (preferentially nervous tissue)
- ☞ Add 1.575 ml of extraction buffer in the tubes.
- ☞ Place the tissue sample in the grinding tubes, close firmly and proceed to the grinding step.
- ☞ Place the tubes in the crown of the homogenizer (Ribolyser). Perform one 45 second agitation cycle at the maximum power of the apparatus (speed 6.5 m/sec).

When grinding is insufficient, another 1 or 2 agitation cycles can be performed.

- ☞ Transfer the extract in Eppendorf tubes (1.5 ml) and centrifuge 5 min at 10.000 rpm.
- ☞ Recover the supernatant and transfer it in new tubes.

Difficulties can be encountered during the grinding step when using dehydrated samples or mechanically resistant tissues (nerves, spleen, tonsyls, lymph nodes ...). If necessary, the grinding step may need to be repeated several times for this type of sample.

### DENATURATION PROTOCOL

After extraction, samples can be denaturated by addition of Urea 6 M (final concentration, diluted in Tris-HCl 10 mM pH 7.4) and heating at 100°C during 10 minutes.  
Before performing the assay, samples should be diluted 1/10, at least in EIA Buffer.



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## REAGENT PREPARATION

The coated plates and reagents are provided ready to use.

### ☞ EIA buffer

Reconstitute one vial with 50 ml of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 month.

### ☞ PrPc Positive Control

Reconstitute the vial with 1 ml of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.  
Stability at -20°C: 1 week.

### ☞ Anti-PrPc AChE tracer

Reconstitute one vial with 10 ml of EIA buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.  
Stability at 4°C: 1 week.

### ☞ Wash buffer

Dilute 1 ml of concentrated wash buffer to 400 ml with distilled or deionized water. Add 200 µl of tween 20 (Use a magnetic stirrer to mix the contents). Stability at 4°C: 1 week.

### ☞ Ellman's Reagent

Five minutes before use, reconstitute with 49 ml of distilled water and 1 ml of concentrated wash buffer. The tube contents should be thoroughly mixed. Stability at 4°C and in the dark: 1 day.

## ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

### **PLATE PREPARATION**

Prepare the wash buffer as indicated in the reagent preparation section. Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet (stored at 4°C). Rinse each well 5 times with the wash buffer (300 µl/well).

Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and dry by inversion on absorbent paper.

### **DISTRIBUTION OF REAGENTS AND SAMPLES**

A plate set-up is suggested on the following page. The contents of each well may be recorded on the sheet provided with the kit.

### **PIPETTING THE REAGENTS**

Note that the first column should be left empty for blanking Ellman's reagent. All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, positive control, samples, tracer, and other reagents.

	12	*	*	*	*	*	*	*	*
	11	*	*	*	*	*	*	*	*
	10	*	*	*	*	*	*	*	*
	9	*	*	*	*	*	*	*	*
	8	*	*	*	*	*	*	*	*
	7	*	*	*	*	*	*	*	*
	6	*	*	*	*	*	*	*	*
	5	*	*	*	*	*	*	*	*
	4	*	*	*	*	*	*	*	*
	3	PC	PC	*	*	*	*	*	*
	2	NSB	NSB	NSB	NSB	NSB	NSB	NSB	NSB
	1	B	B	B	B	B	B	B	B
		<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

B : Blank  
 NSB : Non Specific Binding  
 PC: positive control  
 \* : samples

- ↳ EIA buffer:  
Dispense 100 µl to Non Specific Binding wells.
- ↳ PrPc positive control and samples:  
Dispense 100 µl in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

**INCUBATING AND WASHING THE PLATE**

- ↳ Cover with adhesive film and incubate for 2 hours at room temperature
- ↳ Wash each well five times with the wash buffer (300 µl/well) and then remove the liquid from the wells by inverting the plate. Dry by inversion on absorbant paper and then, dispense 100 µl of anti-PrPc AChE tracer to each well (except blank wells).
- ↳ Cover with adhesive film and incubate for 2 hours at room temperature.

**DEVELOPING AND READING THE PLATE**

Reconstitute Ellman's Reagent as indicated in reagent preparation section. Wash each well five times with the wash buffer (300 µl/well), slightly shake the plate for 5 minutes (with the orbital shaker) and then rewash 5 times with the wash buffer (300 µl/well).  
 Remove the liquid from the wells by inverting the plate.  
 Dry by inversion on absorbent paper.  
 Dispense 200 µl of Ellman's Reagent to the 96 wells. Incubate the plate in darkness at room temperature for 30 minutes. Optimal development is obtained using an orbital shaker. The plate should be read between 405 and 414 nm.

**DATA ANALYSIS**

Make sure that your Plate Reader has substracted the absorbance readings of the blank well (absorbance of Ellman's reagent) from the absorbance readings of the rest of the plate. If not, do it now.

Enzyme Immunoassay Protocol (Volumes are in µl)			
	Blank	Non specific binding	Positive control
Buffer	-	100	-
Positive control	-	-	100
Sample	-	-	-
Cover the plate, incubate at room temperature during 2 hours			
Wash the strips 5 times and remove the liquid from the wells			
Tracer	-	100	100
Cover the plate, incubate at room temperature during 2 hours			
Wash the strips 5 times, slightly shake them 5 min, rewash them 5 times and remove the liquid from the wells			
Ellman's reagent	200	200	200
Incubate the plate with an orbital shaker in the dark at room temperature			
Read the plate between 405 and 414 nm			

## TYPICAL DATA

### EXAMPLE DATA

The following data are for demonstration purpose only. Your data may be different and still correct. Since the concentration of PrPc may vary depending on the homogenised brain zone these results are only indicative. These data were obtained using brain extracts prepared as described in the extraction protocol under the following conditions: 30 minutes developing at room temperature, reading at 414 nm.

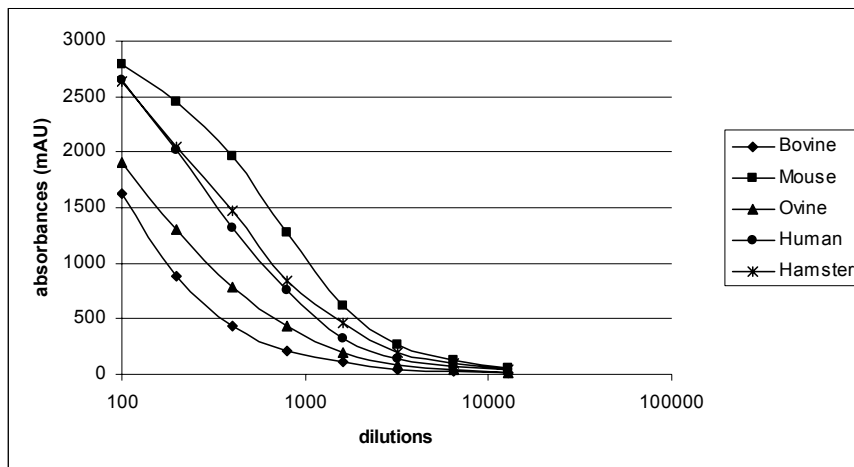
Dilutions of samples	Absorbances (mAU)
Non Specific Binding	23
Positive Control	1022
Bovine extract 1/100	1624
Mouse extract 1/800	1033
Ovine extract 1/200	1132
Human extract 1/400	1029
Hamster extract 1/400	1445

### ACCEPTABLE RANGE

- ☞ Non specific binding < 50 mAU
- ☞ Positive control: between 800 mAU and 1200 mAU.



### BRAIN EXTRACTS DILUTION CURVES



### ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of PrPc has been validated for measuring PrPc in solution after extraction of membranous Prp contained in tissues (preferentially nervous tissues).

- ☞ The cut-off value corresponds to the NSB average (n = 8) plus three standard deviations. This value must be determined for each kind of tissue because NSB varies according to the kind of tissue studied. It can be determined by treatment of the samples with proteinase K (sufficient concentration for extinguishing the signal) and must be tested at the same dilution as the samples.
- ☞ Positive control and extracts intra-assay & inter-assay variations in EIA buffer (n = 30):

	CV %	
	intra-assay	inter-assay
Bovine	3,454	12,569
Mouse	3,802	13,517
Ovine	3,570	5,461
Human	5,553	12,205
Hamster	2,698	9,028
Positive Control	3,148	5,258

### ASSAY TROUBLE SHOOTING

- ☞ Absorbance values too low: incubation in wrong conditions (time or temperature) or reading time too short or PrPc positive control, or samples, or anti-PrP tracer or Ellman's reagent have not been dispensed.
- ☞ NSB value too high: contamination of NSB wells with PrPc positive control, or inefficient washing.
- ☞ High dispersion of duplicates: poor pipetting technique or irregular plate washing.
- ☞ Analyses of two dilutions of a biological sample do not agree: Interfering substances are present.

These are a few examples of trouble shooting that may occur. If you need further explanation, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (contact@spibio.com), and be sure to indicate the batch number of the kit (see outside the box).

SPI-BIO proposes a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39.30.62.60).



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