ENFER TSE Kit
Version 2.0

04J06 (1100 duplicate tests)

Test for in vitro detection of TSE-related PrP^{Sc}.

Method A Manual Homogenisation & Method B Automated Sample Processing

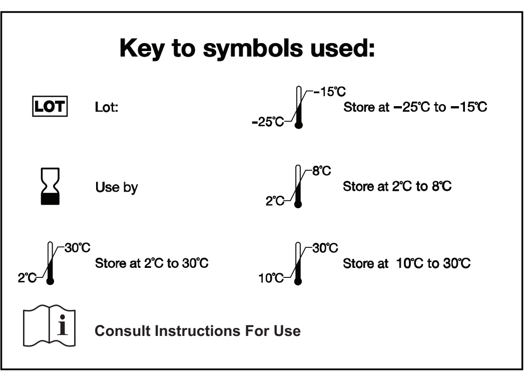
Within the European Union, this test is approved as a rapid test for the BSE testing programme on cattle which is set up in accordance with Regulation (EC) No 999/2001

For in vitro veterinary diagnostic use only

Manufactured by

Enfer Scientific
Unit T, M7 Business Park
Newhall, Naas
Co. Kildare
Ireland
Tel.: +353 459 83800

Enfer Scientific complies with the quality system standard ISO9001



Note Changes Highlighted

The producer of the rapid tests must have a quality assurance system in place agreed by the Community Reference Laboratory (CRL), which ensures that the test performance does not change. The producer must provide the test protocol to the CRL. Sampling tools and modifications to the rapid test or to the test protocol (including sampling) may only be made following advance notification to the CRL and provided that the CRL finds that the modification does not reduce the sensitivity, specificity or reliability of the rapid test. That finding shall be communicated to the Commission and to the National Reference Laboratories

General Information

Transmissible Spongiform Encephalopathies (TSEs) are a group of degenerative neurological diseases. There are a number of examples of TSEs including BSE (Bovine), Scrapie (Ovine), CJD (Human), GSS (Human), Kuru (Human), Transmissible Mink Encephalopathy, Chronic Wasting Disease, Feline Spongiform Encephalopathies and other diseases found in animals such as elk, nyala, greater kudu, gemsbok and tigers. It has also been reported that BSE can be transmitted to mice and pigs under laboratory conditions. This crossing of the species barrier by the infective agent has led to increased concern that transfer to humans could occur.

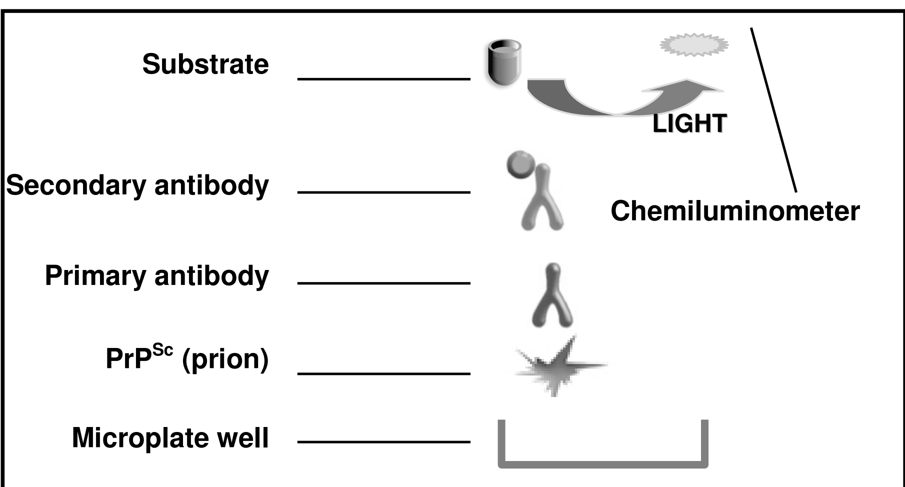
Post-mortems of infected animals reveal a characteristic pattern of vacuolation in the brain tissue due to destruction of neural cells and the deposition of unusual protein fibres, which give the brain a spongy texture. The agent thought to be responsible for TSEs is an infective protein known as a 'Prion'. The prion is an infectious particle believed to comprise of protein only and no nucleic acid. One protein, PrP^{Sc}, has been found to co-purify with infectivity and is the only known component of the characteristic protein fibres deposited in the brain tissue of infected animals.

Intended Use

The Enfer TSE Kit Version 2.0 is a qualitative immunological method for the detection of the unique identifier of Transmissible Spongiform Encephalopathies, the prion protein PrP^{Sc}, in central nervous tissue of cattle. The kit is intended for screening and research purposes only.

Principle of the procedure

A sample of central nervous tissue is collected, transported to the testing laboratory, homogenised under defined conditions and centrifuged. The supernatant is incubated in prepared microplate wells: during this incubation any PrP^{Sc} in the sample is bound to the wells. After a washing step the wells are treated with Enfer Buffer 3. After a second washing step rabbit anti-PrP is added to the well and incubated; if any PrP^{Sc} is present on the well this antiserum will specifically bind to it. After a third washing step goat anti-rabbit IgG conjugated to horseradish peroxidase is added to the wells and incubated; if any rabbit anti-serum is present on the well the conjugate will be bound. After a fourth wash any bound conjugate is detected using a luminogenic substrate for peroxidase. See diagrammatic representation below:



Reagents

Enfer TSE Kit Version 2.0 (04J06) comprising of: 04J06-06 Reagent Pack (1100 test), 04J06-12, Antibody Pack (1100 test)
04J06-26 Buffer Pack (10 Litre), 04J06-31, Wash Pack (1100 test)

Accessory reagent for Enfer TSE Kit Version 2.0 (04J06) 04J06-50 Enfer Buffer 1

Kit Components

Reagent Pack 04J06-06. Contains sufficient material for 1100 tests.

The Reagent Pack is stored at 2 to 8°C. Note storage requirements for the individual components.

Component	Function	Quantity	Storage Requirement
Enfer Buffer 3	Stops Proteinase K reaction and unfolds bound PrP sequence	1 bottle containing 400ml working strength solution	2 to 30 °C, tightly capped
Enfer Wash 1	Rinses off unbound sample	1 bottle containing 1.25kg of powder	2 to 30 °C
Normal Goat Serum	Undiluted serum - Blocks non-specific binding sites	1 microvial containing sufficient material for 1100 tests	2 to 8 °C
Enzyme-conjugate – 2° Ab (goat anti-rabbit)	Undiluted antisera, conjugated to peroxidase, which binds to Anti-PrP – 1° Ab	1 microvial containing sufficient material for 1100 tests	2 to 8 °C
Substrate Solution A	Luminometric substrate for peroxidase when combined with Substrate Solution B	1 bottle containing 250ml of solution	2 to 8 °C
Substrate Solution B	Luminometric substrate for peroxidase when combined with Substrate Solution A	1 bottle containing 250ml of solution	2 to 8 °C, in the dark
Centrifuge Plate	Plate used for centrifugation to pellet out connective tissue	30 96-well plates	2 to 30 °C in sealed bags
Enfer Test Plate	Test plate used for assay	25 96-well plates	2 to 30 °C in sealed bags with the desiccant provided
Peptide Indicator Wells	Peptide-coated wells used to ensure assay is valid	64 wells, provided in 32 pairs	2 to 8 °C in sealed bags with the desiccant provided
Blank Control Reagent	Used as an assay control	1 bottle containing 30ml of a ready to use solution	2 to 30 °C

Antibody Pack 04J06-12. Contains sufficient material for 1100 tests

Component	Function	Quantity	Storage Requirement
Enfer Buffer 2	Contains proteinase K which will digest PrP^{c} preferentially to leave PrP^{Sc}	2 bottles each containing 60ml working strength solution	–25 to –15°C
Anti- PrP – 1° Ab	Undiluted rabbit anti-sera which recognises both PrP^{c} and PrP^{Sc}	Sufficient material for 1100 tests	–25 to –15°C

Buffer Pack 04J06-26. Contains sufficient material for approximately 550 tests. Additional Enfer Buffer 1 can be obtained free of charge by ordering the Accessory Buffer Pack 04J06-50

Component	Function	Quantity	Storage Requirement
Enfer Buffer 1	Homogenisation buffer which liberates both PrP^{c} and PrP^{Sc}	10L working strength solution	10 to 30°C (To minimise precipitation and subsequent dissolution time store Enfer Buffer 1 at between 17 and 28ºC).

Wash Pack 04J06-31. Contains sufficient material for 1100 tests

Component	Function	Quantity	Storage Requirement
Enfer Wash 2	Washes off unbound antibody/reagent	10L of 10x concentrate solution (contains 0.05% Bronidox ®)	10 to 30 °C

Note: Shelf life of kit components is stated on the individual component labels.

Warnings and Precautions

The reagents are solely for *in vitro* veterinary diagnostic use on Bovine samples for Method A and Method B.

For professional use only.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

Perform the sample preparation, sample transfer to the Enfer Test Plate and Wash Step 1 in a Biological Safety Cabinet.

Refer to national TSE safety regulations for country specific precautions.

Health and safety information

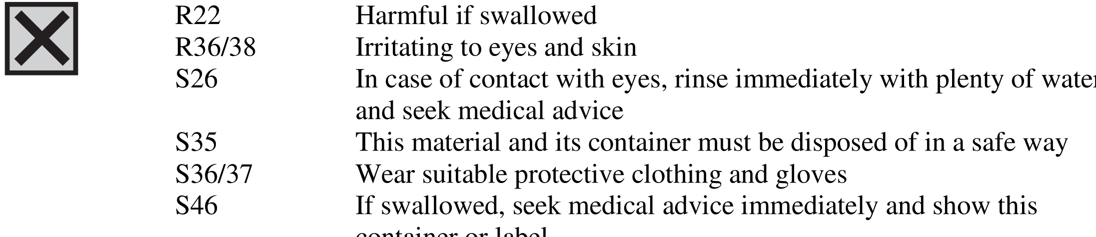
Enfer Buffers 1, 2 & 3 should be handled with care. Avoid contact with skin, eyes and clothes. Do not inhale the reagents, wash immediately with water should contamination occur. Please note hazards identified on individual container labels.

Enfer Buffer 2 contains protease, which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate risk phrases.

Xn		
	R42/43	May cause sensitisation by inhalation and skin contact
	S35	This material and its container must be disposed of in a safe way
	S36/37	Wear suitable protective clothing and gloves
	S46	If swallowed, seek medical advice immediately and show this container or label

Enfer Buffer 3 contains guanidinium chloride, which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate risk phrases.

Xn



R22	Harmful if swallowed
R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S35	This material and its container must be disposed of in a safe way
S36/37	Wear suitable protective clothing and gloves
S46	If swallowed, seek medical advice immediately and show this container or label

Enfer Buffer 1 and the Blank Control Reagent contain methanol, which is classified per applicable European Economic Community (EEC) Directives as toxic (T). The following are the appropriate risk phrases.

T

R10	Flammable
R20/21/22	Harmful through inhalation, in contact with skin and if swallowed
R23/24/25	Toxic by inhalation, in contact with skin and if swallowed
R36/38	Irritating to eyes and skin
R39	Danger of very serious irreversible effects
S16	Keep away from sources of ignition. No smoking
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S35	This material and its container must be disposed of in a safe way
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Information for European Customers: For products not classified as dangerous per European Directive 1999/45/EC, Safety Data Sheets for professional user are available on request.

It is recommended that these reagents and test specimens be handled using established good laboratory working practices.

It is recommended to follow the WHO guidelines for decontamination of work surfaces, liquid and solid waste materials.

Central nervous tissue samples must be disposed of as Specified Risk Material according to local regulations.

See SI No. 146 of 1994 (EU guidelines) for relevant regulations on Safety, Health & Welfare at Work, for an extensive review of recommended safety procedures when working with potentially infectious biological material.

Specimen collection, transport and storage

Refer to Sample Preparation Procedure

No special shipping conditions are required for transportation of samples to the laboratory but when storage of samples is required they can be stored for up to 24 hours at 2 to 8°C or at –25 to –15°C for longer periods. Formalin fixed tissues cannot be used in this assay.

Fixation with formalin for any proposed confirmatory analysis using histopathological or immunohistochemical processes must be done immediately after the samples are taken. Samples for such confirmation must be transported in such a way as not to destroy the sample and must <u>not</u> be frozen, under any circumstances, before formalin fixation is complete.

Analytical Precautions

1. Do not modify the test procedure or substitute reagents from other manufacturers. The Enfer Buffer 1 supplied with this kit may be used interchangeably with reagents from another Enfer TSE Kit. No other reagents may be used interchangeably between kit lots. When using the Tecan Genesis RSP/ Freedom EVO Deep Well Centrifuge Plates must be used. These are supplied separately (order code 01J91-10), and **may** be interchanged between kit lots.
2. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
3. All reagents must be prepared in either clean glass, or polypropylene bottles. Care must be taken to avoid cross contamination of reagents.
4. POLYSTYRENE CONTAINERS MUST NOT BE USED FOR STORAGE OR RECONSTITUTION OF MATERIALS.
5. It is very important that high quality deionised, distilled, or reverse osmosis water is used to reconstitute and dilute reagents as horseradish peroxidase is readily inactivated by pollutants common in laboratory water supplies.
6. Once the assay has been started it must be completed without interruption.
7. Use separate dissection, transfer equipment and disposable tips for each sample to prevent cross-contamination.
8. All reagents must be crystal free, thoroughly mixed and at the temperature of the room (18-30°C) prior to use. Immediately after use return all reagents to the recommended storage temperature.
9. Do not touch or splash the rim of the well with Enzyme-conjugate-2°Ab. Reverse pipetting is recommended for all reagent additions and for transfer of sample from homogeniser bag or ETDS Sample Tube to Centrifuge Plate but **not** for the transfer into the wells of the Enfer Test Plate. Carefully pipette to avoid air bubbles and splashing. Dedicate a pipette for use with the Enfer Substrate Solution.
10. Avoid the use of self-defrosting freezers for the storage of reagents and samples.
11. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
12. Remove plate sealers carefully in a biological safety cabinet, to avoid contamination by aerosols, before the first wash step in the assay procedure.
13. Turn on the incubators at least 30 minutes before they are used to ensure they reach 34°C+/-2°C.
14. Replace the thermal microplate-holder in the incubator slot directly after removing the plate; this ensures it maintains a constant temperature.
15. Ensure that samples do not come into contact with cleaning or disinfecting solutions prior to analysis.

Materials required but not provided

High quality deionised, distilled or reverse osmosis water must be used throughout
2 Skatron Skanwasher® 300 microplate washer*
Thermo LabSystems iEMS incubator/shaker*
Thermo LabSystems Ascent microplate chemiluminescence reader*
Microplate centrifuge capable of 2750g +/-275g
Bottle roller, shaker or magnetic stirrer to help dissolve Enfer Wash 1
Microplate sealers
Multichannel pipettes (5 to 50 µl and 50 to 300 µl)
Micropipettes (5 to 50 µl, 50 to 200 µl, 100 to 1000 µl, 5 ml)
Variable volume reagent dispenser
Top pan balance, accurate to 2 decimal places
Dissection blades or Enfer Sample Cutting Tool (07K34-60)
Weigh boats
Tongue depressors
Glass containers for dilution of the Anti-PrP –1° Ab and the Enzyme – conjugate –2° Ab.
Glass or polypropylene containers for dilution of other reagents

Method A - For Manual Homogenisation:
Interscience Homogeniser or a Seward Stomacher 80*
Homogeniser bags (80 ml capacity with filter)

Method B - For Automated Sample Processing:
ETDS VIII (Europe) (EQP001-1)*
ETDS Homogeniser Assembly (This contains ETDS Sample Tube, Grinding Shaft and Disposable Cap) (01J90-20)*
Homogeniser Rack (EQP002-1)*
Tecan Genesis RSP/ Freedom EVO (01J91-01)*
Deep Well Centrifuge Plate (01J91-10)*

*Apparatus from specified manufacturers is essential for running the assay. These materials/instruments can be purchased through your local representative.

Instrument set-up parameters

The following parameters must be programmed into the recommended instruments. Other instruments have not been validated.

Washer

2 separate washers are required for running this test.

Programmed settings for both protocols:

Air pressure: 0.25 bar
Volume/Flow rate, adjustment offset >>⑨v : 1.00
Aspirate position – set to completely empty well, usually between 3.00mm and 4.00mm
Dispense position – set to give a positive meniscus, usually close to 0.00mm

Protocol 1 (used with Enfer Wash 1 and washer 1. This wash must be performed in a biological safety cabinet.)

Steps:

#	1	Aspirate	6 seconds
#	2	Dispense	300 µl
#	3	Soak	5 seconds
#	4	Aspirate	4 seconds
#	5	Wash	5 seconds
#	6	Soak	5 seconds
#	7	Aspirate	3 seconds
#	8	Wash	2.5 seconds
#	9	Soak	5 seconds
#	10	Aspirate	2 seconds
#	11	Wash	2 seconds
#	12	Soak	5 seconds
#	13	Aspirate	5 seconds
#	14	End Wash	

Protocol 2 (used with Enfer Wash 2 and washer 2)

Steps:

#	1	Aspirate	4 seconds
#	2	Wash	3 seconds
#	3	Soak	5 seconds
#	4	Aspirate	2 seconds
#	5	Wash	3 seconds
#	6	Soak	5 seconds
#	7	Aspirate	2 seconds
#	8	Wash	3 seconds
#	9	Soak	5 seconds
#	10	Aspirate	2 seconds
#	11	Wash	2 seconds
#	12	Soak	5 seconds
#	13	Aspirate	4 seconds
#	14	End Wash	

Shaking incubator: shake value 5, 34°C.

Chemiluminometer: plate acceleration: 10; settle delay: 100; filter: none; measurement type: single; integration time: 300; lag time: 30 seconds; measurement count: 1; photomultiplier (PMT) voltage: default; plate type: 96 well; scale factor: ~8

ETDS VIII: set to homogenise for 10 seconds

Tecan Genesis RSP/ Freedom EVO: set to transfer 350 µl of sample from ETDS Sample Tube to Deep Well Centrifuge Plate, deliver 20 µl per well of Enfer Buffer 2 to the Test Plate and transfer 100 µl of sample from the Deep Well Centrifuge Plate to the Test Plate. All volumes must be accurate to 10%. Contact your local representative for protocols.

Preparation of tissue controls

Negative Tissue Control

Use of a negative tissue control is recommended when routine screening samples are not being tested e.g. during test evaluations where the majority of samples may be positive or dilutions of positive tissues; during repeat testing of initial reactive/suspect positive samples or for investigational purposes.

Prepare a slice of TSE-negative CNS tissue in the same manner as a normal sample and process according to the standard immunoassay protocol below. Samples of TSE-negative CNS tissue can be stored frozen at -25°C to -15°C. If frozen samples are used be sure to allow sample to come to room temperature (18-30ºC) prior to processing in Enfer Buffer 1. Once thawed, negative tissue control must not be re-frozen and must be used on the day it is thawed.

Positive Tissue Control

Peptide Indicator Wells provided in the kit must be used, however a positive tissue control may also be used**.**

Intact tissue stored at -15°C or below should be used. Contact your local representative if homogenisation protocol is required.

BSE positive tissue is infectious and must be handled following strict safety procedures.

Positive and negative tissue controls must be allowed to come to room temperature (18-30°C) before homogenisation in Enfer Buffer 1.

Sample Preparation Procedure

Sampling and laboratory testing must follow the Regulation (EC) No 999/2001 Annex X Chapter C which refers in terms of collection of samples to the latest edition of the ‘‘Manual of Standards for Diagnostic Tests and Vaccines of the International Office of Epizootic Disease (OIE) stating: ‘The preferred sample for immunoassay should be at, or as close to the Obex as possible, but no further than 1.5 cm anterior to, the Obex.’’ See figure 1, appendix 1.

A new blade or Enfer Sample Cutting Tool, tongue depressor, weighing boat and homogeniser bag or ETDS Sample Tube must be used for each sample to prevent cross contamination.

Label an ETDS Sample Tube or homogeniser bag with the identity of the sample it will contain, in the case of the homogeniser bag the label now indicates the front.

Using either a blade or the Enfer Sample Cutting Tool, take a sample of CNS tissue that includes grey matter. The sample should weigh approximately 1.0g; use no less than 0.5g and no more than 2.0g* of tissue to prepare the homogenate. The weight of the sample needs to be determined. (*Samples greater than 1.6g must be processed using more than one ETDS Sample Tube.)

For autolysed samples between 1.5g and 2.0g of tissue must be used – the Enfer Sample Cutting Tool is not suitable for this type of sample. ***See Appendix 1 for further details on the use of the Enfer Sample Cutting Tool.***

If the sample weighs less than 0.5g a fresh sample must be taken.

Note: After sample collection, a complete hemi-section of the brain stem with an intact Obex region must remain available for confirmatory testing.

Method A - Manual Homogenisation

Weigh the sample and place it ***in front of*** the filter in the homogeniser bag, ensuring that the sample is pushed to the bottom of the bag. Squash the sample between thumb and forefinger to aid subsequent homogenisation.

Add the required quantity (12.5 ml of buffer per 1 g of tissue, see Table 1) of Enfer Buffer 1 to the homogeniser bag. The buffer dispenser must not come into contact with the bag to prevent cross contamination of samples.

Homogenise the sample for 2 minutes at speed setting 'high' in a stomacher. Ensure that the tissue sample has been broken down and a clear light brown solution can be seen behind the filter. The assay will be affected if this step is not done correctly. A maximum of two sample bags may be used in the stomacher at any one time.

After homogenisation the samples must be left in the homogeniser bag for between five and ten minutes to allow the bubbles to subside.

Method B - Automated Homogenisation

Weigh the sample and place it inside the outer tube of an unassembled ETDS Sample Tube. To assemble, insert a grinding shaft to sandwich the sample between the grinding surfaces and securely snap the disposable cap on.

Place ETDS Sample Tubes into the Homogeniser Rack taking care to ensure correct alignment. Add the required quantity (12.5 ml of buffer per 1 g of tissue, see Table 1) of Enfer Buffer 1 to each ETDS Sample Tube. Place Homogeniser Rack in position in ETDS VIII and homogenise for 10 seconds.

After homogenisation the samples must be left in the ETDS Sample Tube for between five and ten minutes to allow the bubbles to subside.

For screening purposes do not store homogenised samples, use within 3 hours of preparation.

For confirmatory testing only, where local procedures allow, homogenates must be stored at room temperature and can be re-tested for up to 7 days. Extreme care must be used when interpreting these results as it is known that there is a decrease in signal over time and low positive results at the screening laboratory could become negative. Re-testing must include a fresh cut of tissue to be analysed by ELISA and Western Blot and/or IHC.

Table 1: Sample Dilutions in Enfer Buffer 1 - To ensure satisfactory Assay Performance Enfer Buffer 1 must be at the volumes stated below +/- 5%.

Sample Weight (g)	Volume of Enfer Buffer 1 (ml)
0.51 to 0.55	6.88
0.56 to 0.60	7.50
0.61 to 0.65	8.13
0.66 to 0.70	8.75
0.71 to 0.75	9.38
0.76 to 0.80	10.00
0.81 to 0.85	10.63
0.86 to 0.90	11.25
0.91 to 0.95	11.88
0.96 to 1.00	12.50
1.01 to 1.05	13.13

Sample Weight (g)	Volume of Enfer Buffer 1 (ml)
1.06 to 1.10	13.75
1.11 to 1.15	14.38
1.16 to 1.20	15.00
1.21 to 1.25	15.63
1.26 to 1.30	16.25
1.31 to 1.35	16.88
1.36 to 1.40	17.50
1.41 to 1.45	18.13
1.46 to 1.50	18.75
1.51 to 1.55	19.38
1.56 to 1.60	20.00

Preparation of reagents

High quality deionised, distilled or reverse osmosis water must be used for the preparation of reagents.

Component	Method	Storage of Prepared Reagents
Enfer Wash 1 Solution	1. Add Enfer Wash 1 solid to water at a ratio of 50 g per litre of water. 2. Allow to dissolve on a rotating bottle roller, shaker or a magnetic stir-plate for 10 min, or until in solution.	1 month at 10 to 30°C
Enfer Wash 2, Working Strength	1. Ensure buffer is crystal free and thoroughly mixed prior to use. If crystals are present, incubation at 30°C is recommended; do not exceed 37°C. 2. Dilute Enfer Wash 2 concentrate 1 in 10 in water. 3. Mix thoroughly E.g. For 4 L of working strength Enfer Wash 2, add 400 ml Enfer Wash 2 concentrate to 3600 ml of water	2 weeks at 10 to 30°C 1 month at 2 to 8°C
Enfer Buffer 1	1. Ensure buffer is crystal free and thoroughly mixed prior to use. If crystals are present, incubate at between 17 and 28°C. Stirring the buffer with an overhead stirrer will aid dissolution.	As labelled
Centrifuge Plate	1. Ensure plate is at room temperature (18-30°C) before starting the assay 2. Return unused plates to the storage bag	As labelled
Enfer Buffer 2	1. Thaw completely 2. Mix well. 3. Solution is then ready to use	As labelled with up to 15 freeze-thaw cycles.
Enfer Test Plate	1. Ensure plate is at room temperature (18-30°C) before starting the assay 2. The test plate may be dismantled, return unused wells to the storage bag with desiccant.	As labelled
Peptide Indicator Wells	1. Ensure Peptide Indicator wells are at room temperature (18-30°C) before starting the assay 2. Return unused wells to the storage bag containing desiccant	As labelled
Blank Control	1. Ensure buffer is crystal free and thoroughly mixed prior to use. If crystals are present, incubate at between 17 and 28°C.	As labelled
Enfer Buffer 3	1. Solution is ready to use. Ensure the solution is at room temperature (18-30°C) before use.	As labelled

Component	Method	Storage of Prepared Reagents
Anti-PrP – 1° Ab, Working Strength Requires: 1. Working strength Enfer Wash 2 2. Anti-PrP-1°Ab 3. Normal Goat Serum (NGS)	Prepare only the required volume for the number of tests to be carried out. 2 ml of working strength 1°Ab is required for 8 wells. 20 ml of working strength 1°Ab is required for 1 plate. 1. Thaw Anti-PrP – 1° Ab completely and mix by inversion 2. Dilute NGS 1 in 500 in working strength Enfer Wash 2 3. Dilute Anti-PrP – 1° Ab 1 in 500 in working strength Enfer Wash 2 plus NGS 4. Mix by inversion. Invert a minimum of 8 times. E.g. To 20 ml of working strength Enfer Wash 2 solution add 40µl of NGS and 40µl of Anti-PrP – 1° Ab **Notes:** Return unused NGS to 2 to 8°C storage and Anti-PrP – 1° Ab to –15 to –25°C after use	Store at 2 to 8°C and use on day of preparation.
Enzyme-conjugate – 2° Ab, **Working Strength** Requires: 1. Working strength Enfer Wash 2 2. Enzyme-conjugate – 2° Ab	Prepare only the required volume for the number of tests to be carried out. 2 ml of working strength 2°Ab is required for 8 wells. 20 ml of working strength 2°Ab is required for 1 plate. 1. Dilute Enzyme-conjugate – 2° Ab 1 in 1500 in working strength Enfer Wash 2 2. Mix by inversion. Invert a minimum of 8 times. E.g. To 20 ml of working strength Enfer Wash 2 solution add 13.3 µl of Enzyme-conjugate – 2° Ab **Notes:** Return unused Enzyme-conjugate – 2° Ab to 2 to 8°C storage after use	Store at 2 to 8°C in the dark and use within 2 hours of preparation.
Substrate, **Working Strength**	Prepare only the required volume for the number of tests to be carried out. Prepare the Substrate solution at least one hour before use to allow it to come to room temperature (18-30°C). 2 ml of working strength substrate is required for 8 wells. 20 ml of working strength substrate is required for 1 plate. 1. Add an equal volume of Substrate Solution A to Substrate Solution B 2. Mix by inversion. Invert a minimum of 8 times. E.g. Add 10 ml of Substrate Solution A to 10 ml of Substrate Solution B **Notes:** a) Do not cross contaminate Substrate A and B solution bottles; use separate pipettes and do not interchange caps. b) Return unused Substrate solutions A and B to 2 to 8°C storage directly after preparing working strength Substrate.	Store at 18-30°C in the dark and use on the day of preparation.

Immunoassay Procedure – *(Also refer to Appendix 2)*

*All reagents including wash solutions, Enfer Test Plates, Peptide Indicator Wells and Centrifuge Plates must be at the temperature of the room (between 18 and 30°C) before starting the assay. Enfer Buffer 1, Wash 2 concentrate and Blank Control Reagent must be at the temperature of the room (between 18 and 30°C) and **crystal free** before starting the assay. If crystals are present, incubate at between 17 and 28ºC for the Enfer Buffer 1 and Blank Control Reagent and for Wash 2 concentrate incubation at 30ºC is recommended. Stirring the buffer with an overhead stirrer will aid dissolution. Storing these buffers in a transparent container is highly recommended so that any crystals are easily visible.*

1) Manual Pipetting

Dispense 180 µl Blank Control Reagent in quadruplicate, 180 µl of each sample in duplicate and 180 µl controls, if tested, in duplicate onto the **Centrifuge Plate**. The centrifuge plate provided with the kit **must be used**. Do not add any samples or controls to positions A1 and A2

Always pipette from behind the filter in the homogeniser bag. Take great care not to allow the pipette to be contaminated on the outside by material from within the homogeniser bag. Ensure that only the disposable tip is inserted into the bag.

or

1*) Automated Pipetting

Dispense 350 µl Blank Control Reagent in quadruplicate, 350 µl of each sample in duplicate and 350 µl controls, if tested, in duplicate onto the **Deep Well Centrifuge Plate**. Do not add any samples or controls to positions A1 and A2

If using the ETDS Sample Tube, pipette from the hollow grinding shaft. Ensure that only the disposable tip is inserted into the tube.

2) Cover the plate with a plate sealer.

3) Centrifuge the plate at 2750 g for 5 minutes at 18 to 30°C.

4*) Add 20 µl of Enfer Buffer 2 to all the wells of the **Enfer Test Plate**. It is very important that this buffer is pipetted to the very bottom of the wells: it has a tendency to stick to the sides of the wells and this must be prevented.

5*) Remove the plate sealer and transfer 100 µl of each centrifuged sample, Blank Control Reagent and tissue controls to the corresponding position on the **Enfer Test Plate** containing Enfer Buffer 2. Handle the centrifuge plate very carefully to avoid disturbing the pellet and take care not to transfer any solid material to the Enfer Test Plate.

6) Cover the plate with a plate sealer.

7) Incubate the plate, shaking, for 60 minutes at 34°C.

8) Remove the plate sealer and wash the plate using Enfer Wash 1 solution and wash protocol 1.

9) Invert the plate on a wad of tissue and tap it to remove any remaining liquid.

10) Add 150 µl of Enfer Buffer 3 to all the wells.

11) Cover the plate with a plate sealer.

12) Incubate the plate, shaking, for 15 minutes at 34°C.

13) Remove the plate sealer and wash the plate using Enfer Wash 2 solution and wash protocol 2.

14) Invert the plate on a wad of tissue and tap it to remove any remaining liquid.

15) Remove positions A1 and A2 from the Enfer Test Plate and replace with Peptide Indicator Wells.

    It is extremely important to ensure that these wells are inserted properly – the top of the wells MUST be flush with the tops of all the other wells on the plate.

16) Dispense 150 µl of working strength Anti-PrP – 1°Ab into each well.

17) Cover the plate with a plate sealer.

18) Incubate the plate, shaking, at 34°C for 40 minutes.

19) Remove the plate sealer and wash the plate using Enfer Wash 2 solution and wash protocol 2.

20) Invert the plate on a wad of tissue and tap it to remove any remaining liquid.

21) Dispense 150 µl of working strength Enzyme-conjugate– 2°Ab onto the plate.

22) Cover the plate with a plate sealer.

23) Incubate the plate, shaking, at 34°C for 30 minutes.

24) Remove the plate sealer and wash the plate using Enfer Wash 2 solution and wash protocol 2.

25) Invert the plate on a wad of tissue and tap it to remove any remaining liquid.

26) Add 150 µl of working strength Substrate Solution to the plate

27) Cover the plate with a plate sealer.

28) Incubate the plate, shaking, at 34°C for 10 minutes.

29) Remove the plate sealer and read the light signal immediately using a chemiluminometer.

*The Tecan Genesis RSP/ Freedom EVO automated pipetting system can be used for these steps. Contact your local representative for validated protocols.

To ensure satisfactory performance, incubation temperatures must be 34°C +/- 2°C.

With the exception of sample dilution in Enfer Buffer 1 all volumes pipetted must be within +/- 10% of the stated volume, the following are the stated volumes with the allowed range in parenthesis, 100µl (90 to 110µl), 20µl (18 to 22µl), 150µl (135 to 165µl) &350µl (315 to 385µl). The volume of Enfer Buffer 1 added must be within +/-5% of the values stated in table 1.

Incubation times may not be reduced and must be accurate to within +10%. The centrifugation speed must be within 2750 g +10%, with a minimum centrifugation time of 5 minutes not to exceed 10 minutes.

Validation of Test Performance

The control results must be validated before the sample results can be interpreted.

Determine the mean luminescence of Peptide Indicator Wells, Positive and Negative Controls (if applicable) and calculate the median value for the Blank Control Reagent.

Acceptable Range of Control Results:

The values given are for measurements made on an Enfer recommended chemiluminometer.

Blank Control Reagent

To calculate the median value of the Blank Control Reagent, arrange the four Light Unit values in ascending numerical order. The median is the arithmetic average of the two middle values.

The median of Blank Control Reagent replicates must be below 4.0 LU.

Peptide Indicator Well:

See the value for the provided lot of Peptide Indicator Wells.

The mean value must be equal to or above the limit supplied with the Peptide Indicator Wells (after subtraction of the median blank value).

Negative Tissue Control

If negative control is run, the mean must be less than 5.5 LU after subtraction of the median blank reading.

If the above criteria are not met, the EIA run is invalid and must be repeated.

Sensitivity

The threshold light signal for a suspect positive determination is 5.5 LU (after subtraction of the median blank reading) as measured on an Enfer recommended chemiluminometer. All samples giving signals greater than or equal to 5.5 LU (after subtraction of the median blank reading) in one or both duplicate wells must be considered initially reactive and must be retested in duplicate, starting from the tissue. A sample is considered positive when the retesting results give a positive signal in one or both wells

Samples and the corresponding tissue giving positive test results should be sent to the NRL for confirmation.

Limitations of the Procedure

Enfer Scientific complies with the quality system standard ISO9001.

As with any biological test, this test may give a false positive or a false negative result owing to local conditions. A test should be interpreted in the context of all available clinical, historical and epidemiological information relevant to the animal(s) under test. Further confirmatory testing may be required in certain circumstances.

A negative result with a qualitative immunological method does not preclude the possibility of infection with the prion protein PrP^{Sc}.

These performance data were obtained using the procedure described. Any change or modification of the procedure might affect the results.

Results obtained for TSE positive material may vary as prion distribution in tissue varies and stability of stored tissue cannot be guaranteed.

Responsibility for test interpretation and consequent animal husbandry decisions rests solely with the user and any consulting veterinarian and appropriate animal health advisors or authorities. Enfer Scientific accepts no responsibility for any loss or damage, howsoever caused, arising out of the interpretation of test results.

Disclaimer & Reservation of Rights

Enfer Scientific gives no warranty of any kind, whether expressed or implied, in regard to the carrying out of the Enfer TSE Kit Version 2.0, or for the stability and storage of the Enfer kit, or for the procedure used. Without prejudice to the foregoing, Enfer Scientific disclaims all responsibility for merchantability and fitness for use after it leaves Enfer Scientific. Enfer Scientific shall not be liable, under any circumstances, for damages, direct or consequential.

Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	**P**	**P**	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	**B**	**B**	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	**B**	**B**	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	**N**	**N**	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

P = Peptide Indicator Wells

B = Blank Control Reagent

N = Negative Tissue Control (if applicable)

S = Test Samples in duplicate

Bronidox ® is not a trademark of Enfer.

C134J06GB

November 2010

Appendix 1

Directions for sampling tissue using the Enfer Sample Cutting Tool (07K34-60)

Materials required:

Enfer Sample Cutting Tool (referred to as 'Cutting Tool' below)

Tissue samples

Disposable weigh boats or dishes for samples

Disposable spatulas or tongue depressors

ETDS Sample Tubes or homogeniser bags

Note: This method should only be performed by experienced operating technicians

Method:

1. Use a new Cutting Tool, weigh boat and tongue depressor for each sample to avoid cross contamination.
2. Place the sample in a dish and identify the area you wish to sample. This will be one side of the Obex, as shown in Figure 1.
3. Place the Cutting Tool over the area to be sampled, with the bevelled cutting edge of the tool against the tissue.
4. Use the disposable spatula or tongue depressor to anchor the tissue in place. Cut the tissue by applying gentle downwards pressure on the Cutting Tool whilst twisting the tool clockwise and anti-clockwise through approximately quarter of a turn. See Figure 2.
5. The tool will cut a cylindrical piece of tissue. See Figure 3. The tissue normally remains inside the Cutting Tool.
6. To transfer the tissue to an ETDS Sample Tube tap the rim of the Cutting Tool against the rim of the ETDS Sample Tube; this is generally sufficient to dislodge the tissue, if the tissue remains inside the Cutting Tool dislodge it by pressing a spatula through the side openings (Note: Tare the balance with the empty outer tube before adding the sample).

OR To transfer the tissue to a homogeniser bag, place the end of the Cutting Tool containing the tissue inside the homogeniser bag and dislodge the tissue by pressing a spatula through the side openings in the cutting tool (Note: Tare the balance using the empty bag before adding the sample). Ensure the sample is at the bottom of the homogeniser bag before processing further.

Note: After sample collection, a complete hemi-section of the brain stem with an intact Obex region must remain available for confirmatory testing.

Figure 1.

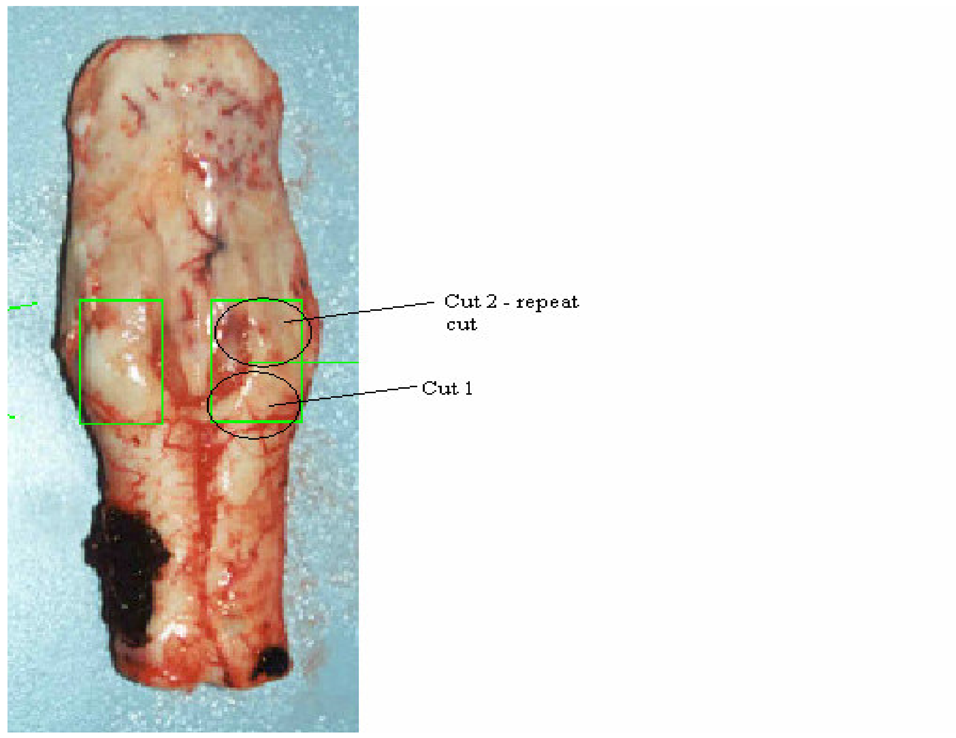


Figure 2.

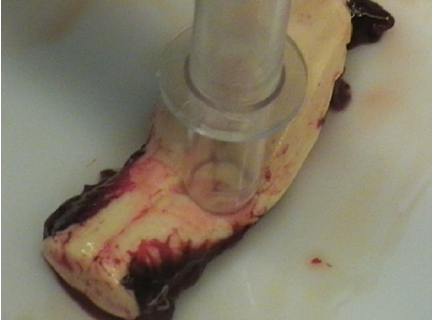
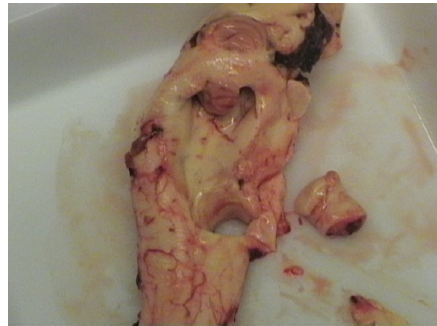


Figure 3.



N<u>ote</u>: The Enfer Sample Cutting Tool is not suitable for autolysed samples

Appendix 2 - Flow chart for manual and automated Immunoassay procedure

Cut Sample (0.5- 2.0g)

Dissection Blade

Enfer Sample Cutting Tool

Homogenise in Enfer Buffer 1

Stomacher - **2 mins** 'High' Speed (Method A)

ETDS VIII Homogeniser **- 10 secs** (Method B)

Add Sample & Controls to Centrifuge Plate

180µl/well, manually to Centrifuge Plate provided with kit

350µl/well to the Deep Well Centrifuge Plate using Tecan Genesis RSP/ Freedom EVO

Add Enfer Buffer 2 to Test Plate (20µl/well)

Manually

Using Tecan Genesis RSP/ Freedom EVO

Transfer sample Enfer (100µl) to Test Plate

Manually from Centrifuge Plate to Test Plate

From Deep Well Centrifuge Plate, using Tecan Genesis RSP/ Freedom EVO

Add Enfer Buffer 3

Add 150µl Enfer Buffer 3/well

Incubate* 60 mins

Enfer Wash 1**

Prepare working strength Anti-PrP-1°Ab

Add Anti-PrP-1°Ab

Add 150µl Anti-PrP-1°Ab/well

Incubate* 15 mins

Enfer Wash 2**

Prepare working strength Enzyme-conjugate-2°Ab

Add Enzyme-conjugate 2°Ab

Add 150µl Enzyme-conjugate-2°Ab/well

Incubate* 40 mins

Enfer Wash 2**

Prepare working strength Substrate Solution, A:B - 1:1

Add Substrate Solution

Add 150µl Substrate Solution/well

Incubate* 30 mins

Enfer Wash 2**

Read Plate

Incubate* 10 mins

Immediately read using Chemiluminometer

* All Incubations are performed at 34°C ** Prepare working strength wash solution according to instructions on page 12 of this booklet.