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Prion Protein Extraction in Animal Tissues

URI282 – Animal Infectiology and Public Health National Institute of Agronomic Research of Nouzilly

CONTEXT

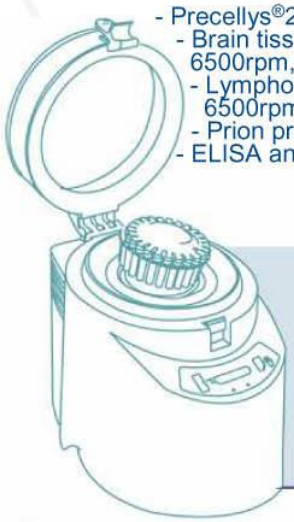
In TSE, the preparation of animal tissues is an important step for the detection of the pathological prion protein. Brain and spinal cord tissues, lymphoid tissues (tonsils, lymph nodes, spleen…) are frozen at -80°C. The samples are suspended in a buffer, and protein extraction is followed by ELISA analysis and Western Blot Analysis.

MATERIAL

- Precellys®24
- Precellys® kit CK14 (small ceramic beads)
- Sample : 50 – 150 mg of brain and lymphoid tissues
- Buffer : glucose 5% (500 - 1500µl) added after grinding

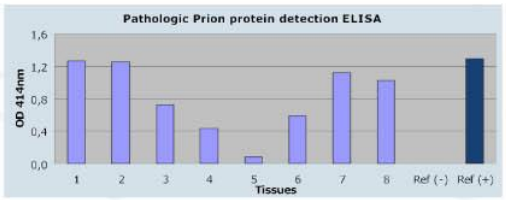
PROTOCOL

- Precellys®24 parameters :
  - Brain tissues :
    6500rpm, 2x30 sec.,15 sec. break
  - Lymphoid tissues :
    6500rpm, 3x30 sec., 20 sec. break
  - Prion protein extraction
  - ELISA analysis and Western Blot

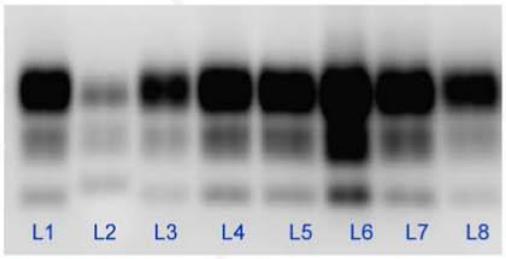


RESULTS

After prion protein extraction, the pathologic protein is detected and quantified by using specific antibodies through an ELISA test, and the pattern of the glycozylated protein is analyzed by Western Blot.



ELISA analysis



Lanes 4-6 : two positive references
Lanes 1-3 : BSE or Scrapie infected brain tissues
Lanes 5,7,8 : BSE infected lymphoid tissues

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CONCLUSION

The Precellys®24 and kit CK14 allow the homogenization of a large range of animal tissues. The preparation optimized the extraction for quantification of the prion protein.

For more details, please contact precellys@bertin.fr or visit our website

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