Extraction of Aβ from Brain for Aβ ELISA

This protocol is a method to extract Aβ from brain for application to Aβ ELISA

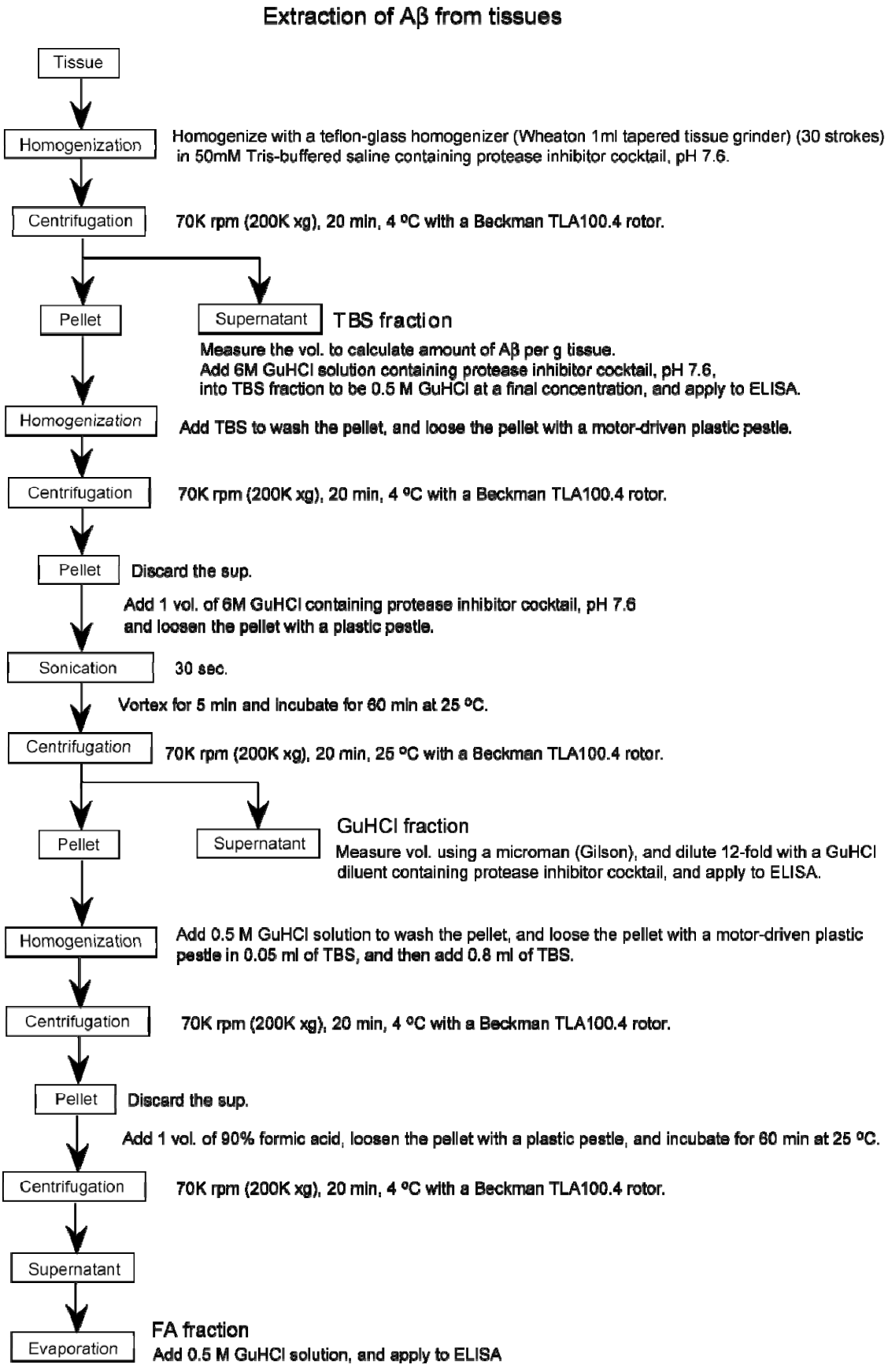
Reagents

- 50mM Tris-buffered saline containing protease inhibitor cocktail, pH 7.6.
- 6M GuHCl solution (6M GuHCl/50mM Tris-HCl, protease inhibitor cocktail, pH 7.6) Store at -30 ^{o}C.
- 90% formic acid
- GuHCl diluent (20mM phosphate, 0.4M NaCl, 2mM EDTA, 10% Block Ace, 0.2% BSA, 0.05% NaN3, 0.075% CHAPS, protease inhibitor cocktail, pH 7.0)

Equipment

- Teflon-glass homogenizer (Wheaton 1ml tapered tissue grinder)
- Beckman Optima TL Ultracentrifuge, TLA100.4 rotor
- Motor-driven plastic pestle
- Evaporator

Procedure


Extraction of Aβ from tissues
Tissue
Homogenization
Homogenize with a teflon-glass homogenizer (Wheaton 1ml tapered tissue grinder) (30 strokes) in 50mM Tris-buffered saline containing protease inhibitor cocktail, pH 7.6.
Centrifugation
70K rpm (200K xg), 20 min, 4 ºC with a Beckman TLA100.4 rotor.
Pellet
Supernatant
TBS fraction
Measure the vol. to calculate amount of Aβ per g tissue.
Add 6M GuHCl solution containing protease inhibitor cocktail, pH 7.6, into TBS fraction to be 0.5 M GuHCl at a final concentration, and apply to ELISA.
Homogenization
Add TBS to wash the pellet, and loose the pellet with a motor-driven plastic pestle.
Centrifugation
70K rpm (200K xg), 20 min, 4 ºC with a Beckman TLA100.4 rotor.
Pellet
Discard the sup.
Add 1 vol. of 6M GuHCl containing protease inhibitor cocktail, pH 7.6 and loosen the pellet with a plastic pestle.
Sonication
30 sec.
Vortex for 5 min and incubate for 60 min at 25 ºC.
Centrifugation
70K rpm (200K xg), 20 min, 25 ºC with a Beckman TLA100.4 rotor.
Pellet
Supernatant
GuHCl fraction
Measure vol. using a microman (Gilson), and dilute 12-fold with a GuHCl diluent containing protease inhibitor cocktail, and apply to ELISA.
Homogenization
Add 0.5 M GuHCl solution to wash the pellet, and loose the pellet with a motor-driven plastic pestle in 0.05 ml of TBS, and then add 0.8 ml of TBS.
Centrifugation
70K rpm (200K xg), 20 min, 4 ºC with a Beckman TLA100.4 rotor.
Pellet
Discard the sup.
Add 1 vol. of 90% formic acid, loosen the pellet with a plastic pestle, and incubate for 60 min at 25 ºC.
Centrifugation
70K rpm (200K xg), 20 min, 4 ºC with a Beckman TLA100.4 rotor.
Supernatant
Evaporation
FA fraction
Add 0.5 M GuHCl solution, and apply to ELISA

References

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