Brain Homogenate Preparation

Brain homogenate prep buffers (**make fresh):

1. Brain Homogenation Buffer (BHB):

1X PBS (pH = 7.4) with 1 mM EDTA	19.3 mL
5M NaCl	0.6 mL
Triton X 100	0.1 mL
Complete Protease Inhibitor w/o EDTA (Rosche, 11836170001)	1 Tablet

*gently mix at 4°C until PI Tablet dissolves and then put on ice until use.

Steps:

1.) Perfuse normal or scrapie-affected Syrian golden hamsters with ice cold 1X PBS-EDTA:

NaCl	8g
KCl	0.2g
Na_2PO_4	1.44g
KH_2PO_4	0.24g
+5mM EDTA	

*pH to 7.4 with HCl and QS to 1L

Note: Perfused tissue may be preferential because we are aware on some level of assay inhibition caused by contaminating blood. However, perfusion is not absolutely necessary for Brain Homogenate preparation.

2.) Extract hamster brain with clean tools and flash freeze with liquid nitrogen
3.) Store brains at -80°C
4.) Rinse 2mL dounce to remove particulates from packaging or storage.
5.) Weigh brain chunks:

a. Normal Brain Weight: _____

b. Diseased Brain Weight: _____

6.) Use 10% weight per volume of Brain Homogenate Buffer (i.e. 0.1g Brain = 1r[illegible]3HB).
7.) Dounce 10X with loose dounce and 10 times with tight dounce.
   a. Minimize frothing/bubbles
   b. All dounces performed on ice in 50mL tube.

Note: In addition to using a dounce as described here, we have also had success preparing Brain Homogenates for RTQ using Bead Beater and Tissue Grinder protocols.

8.) Spin at 2000Xg for 2min to partially clarify and transfer supernatant to another tube.
9.) Aliquot supe into small (i.e. 12uL) aliquots and put at -80°C for storage.