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# GENERATION OF LYSATE INOCULATION MATERIALS

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**We use this protocol and it's working**

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## Abstract

This protocol details inoculation lysate preparation.

## Attachments

DOCX

1035 - 2660.docx

18KB

## Materials

**Lysis buffer:**

| | A | B |
|---|---|---|
| | Sucrose | 250 mM |
| | HEPES | 20mM |
| | KCl | 10mM |
| | MgCl2 | 1.5mM |
| | EDTA | 2 mM |
| | Protease-inhibitor cocktail | |

## Soluble/Insoluble lysate preparation from harvested brainstem/spinal cord tissue

1 Acquire the tissue from 4-month-old asymptomatic TgA53T (Line G2-3) and end-stage tissue harvest and store at -80 °C prior to use.

2 Weigh the tissue and suspend it in 0.9% sterile saline (1:10 w/vol).

3 Homogenize and centrifuge for 00:05:00 at 3000 x g at 4 °C. The supernatant (S3000) is obtained. 5m

4 Centrifuge S3000 for 00:45:00 at 150000 x g at 4 °C. The supernatant (S150) – Highly soluble fraction is obtained. 45m

5 Wash pellet and resuspend in sterile saline (half of original volume) by sonication (3 × 10 min pulses). Resuspended pellet (P150) – Insoluble fraction is obtained.

5.1 Wash pellet and resuspend in sterile saline (half of original volume) by sonication for 00:00:10 pulses) (1/3). 10s

5.2 Wash pellet and resuspend in sterile saline (half of original volume) by sonication for 00:00:10 pulses) (2/3). 10s

5.3 Wash pellet and resuspend in sterile saline (half of original volume) by sonication for 00:00:10 pulses) (3/3). 10s

## Endoplasmic Reticulum (ER)-enriched fractionation

6 Homogenize freshly harvested tissues (1:10 w/vol) in lysis buffer (refer material section).

7 Centrifuge at 1000 x g and collect the supernatant.

8 Centrifuge at 10000 x g . The pellet containing mitochondria is obtained.

9 Centrifuge supernatant at 100000 x g . The supernatant containing cytosol and pellet containing ER are obtained.