

Mar 20, 2024

GENERATION OF LYSATE INOCULATION MATERIALS

DOI

<https://dx.doi.org/10.17504/protocols.io.4r3l22d2ql1y/v1>

Scott Vermilyea¹

¹University of Minnesota

Team Lee



Jane Balster

ASAP - Team Lee

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.4r3l22d2ql1y/v1>

Protocol Citation: Scott Vermilyea 2024. GENERATION OF LYSATE INOCULATION MATERIALS. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.4r3l22d2ql1y/v1>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: February 21, 2024

Last Modified: May 31, 2024

Protocol Integer ID: 95767

Keywords: ASAPCRN, generation of lysate inoculation material, protocol details inoculation lysate preparation, lysate inoculation material, lysate preparation, preparation, protocol details inoculation, material

Abstract

This protocol details inoculation lysate preparation.

Attachments



[1035 - 2660.docx](#)

18KB

Materials

Lysis buffer:

	A	B
	Sucrose	250 mM
	HEPES	20mM
	KCl	10mM
	MgCl ₂	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. 