Protein extraction from aortas or VSMCs for Western blot

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Abstract

This protocol describes protein extraction steps from mouse aorta or vascular smooth muscle cell sample for Western blot. The lysis buffer use is a SMAD lysys buffer adopted from Beaufort et al (2014).

Citation: Muthi Ikawati Protein extraction from aortas or VSMCs for Western blot. **protocols.io** dx.doi.org/10.17504/protocols.io.nxadfie
Published: 19 Mar 2018

Protocol

Sample preparation

Step 1.

Thaw frozen aorta in a 37°C waterbath, mince on ice, and pool aortas from 3-4 mice in a single microtube.

For VMSCs, wash cells with phosphate-buffered saline (PBS) three times, aspirate the PBS thoroughly.

Lysis buffer

Step 2.

Prepare SMAD lysis buffer:

10 mM Tris-HCl pH 7.6,

1% Triton X-100,

100 mM NaCl,

2 mM EDTA,

10% v/v glycerol

containing 50 mM NaF,

20 mM $Na_4P_20_7$,

2 mM Na_3VO_4,

and 1x protease inhibitor cocktail (Nacalai Tesque)

Protein extraction

Step 3.

Lyse pooled minced aortas or VSMCs in 100 or 150 µl lysis buffer for each microtube (around 30 mg tissue) or 35 mm dish, respectively.

Protein extraction

Step 4.

Use a homogenizer (for aortas) followed by a sonicator to further lyse the tissue.

Use a sonicator to process VSMC samples.

Protein extraction

Step 5.

Centrifuge 15,000 x g for 10 min at 4°C.

TEMPERATURE

4 °C Additional info:

Protein extraction

Step 6.

Transfer supernatant into a new microtube.

Measurement of protein concentration

Step 7.

Measure protein concentration by using Pierce 660nm Protein Assay Reagent. Make a BSA standard curve as well to plot the absorbance into the concentration value.

REAGENTS

Pierce™ 660nm Protein Assay Reagent 22660 by Thermo Fisher Scientific

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Storage

Step 8.

Add 5x SDS loading dye, boil at 95ºC for 3-5 min.

TEMPERATURE

95 °C Additional info:

Storage

Step 9.

Store at -20ºC until used.