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Sample Preparation Protocols for Tissues

Essential sample protocols to prepare tissues and brain synaptosomes for western blot.

Sample preparation is a crucial first step in any western blot (WB). The preparation protocol you use depends on the type of sample you plan to use. For example, the optimal method to extract the same protein from heart versus adipose tissue, will not necessarily be the same one. You also need to consider the subcellular localization of your protein (plasma membrane proteins and nuclear proteins, for example, would need different sample preparation methods) and whether or not the target protein is enriched in specific microdomains like lipid rafts.

Beginning your WB with the proper sample preparation protocol, that considers the nature and location of your protein, is critical if you want to produce robust results.

Below you can find detailed steps for multiple sample preparation methods.

- Enriched Membrane Fraction Preparation
- Lysate Preparation
- Brain Synaptosome Preparation

If you need sample preparation for cells, take a look at our Sample Preparation Protocols for Cell Lines.

Enriched Membrane Fraction Preparation

1. Remove the tissue of interest from the animal and flash freeze it in liquid nitrogen. Store the tissue at -80°C until further use.

2. Place the frozen tissue in 5 volumes of ice-cold Lysis Buffer A.

Lysis Buffer A

Reagent	Concentration
HEPES (pH 7.4)	4 mM
Sucrose	320 mM
EDTA (pH 8)	5 mM

Complete EDTA-free protease inhibitor cocktail (Roche)

3. Homogenize the tissue with a polytron homogenizer.

4. Centrifuge the homogenate for 10 minutes at 2,000 x g at 4°C. Discard the large debris.

5. Transfer the supernatant to a clean tube and resuspend the pellet in 2 volumes of Lysis Buffer A and rehomogenize.

6. Centrifuge the homogenate for 10 minutes at 2,000 x g at 4°C. Combine the supernatant with that from step 5.

7. Centrifuge the supernatants (from steps 5 and 6) for 1 hour at 100,000 x g at 4°C.

8. Discard the supernatant. Resuspend the pellet (that contains tissue membranes) in 2 volumes of Lysis Buffer A and briefly homogenize with a polytron homogenizer.

9. Measure the protein concentration using the Bradford method. Adjust the protein concentration to 4 mg/ml with Lysis Buffer A.

10. Store protein samples at -80°C until further use.

Lysate Preparation

1. Remove the tissue/organ of interest from the animal and flash freeze it in liquid nitrogen. Store the tissue/organ at -80°C until further use.

2. Place the frozen tissue in 5 volumes of ice-cold Lysis Buffer B.

Lysis Buffer B

Reagent	Concentration
Tris (pH 7.4)	50 mM
Triton X-100	1%
EDTA (pH 8)	5 mM

Complete EDTA-free protease inhibitor cocktail (Roche)

3. Homogenize the tissue with a polytron homogenizer.

4. Rotate the sample for 30 minutes at 4°C.

5. Centrifuge the homogenate for 1 hour at 100,000 x g at 4°C.

6. Transfer the supernatant to a clean tube and measure the protein concentration using the Bradford method. Adjust the protein concentration to 4 mg/ml with Lysis Buffer B. Store the lysate at -80°C until further use.

Sample Preparation for Brain Synaptosomes

This brain synaptosomal preparation is commonly called the “P2” protocol. This protocol enriches the synaptosomal fraction of the brain and generally enables easy detection of synapse-enriched proteins for WB.

1. Remove brains and flash-freeze in liquid nitrogen. Keep brains at -80°C until further use.
2. Resuspend the frozen brains in 5 volumes of ice-cold Lysis Buffer C. Work on ice.

Lysis Buffer C

Reagent	Concentration
HEPES (pH 7.4)	4 mM
Sucrose	320 mM
EDTA (pH 8)	5 mM
Phenylmethylsulfonyl fluoride (PMSF)	1 mM
Complete EDTA-free protease inhibitor cocktail (Roche)	

3. Homogenize brains with a Polytron homogenizer.
4. Centrifuge homogenates at 700 x g for 10 minutes at 4°C.
5. Transfer resulting supernatant to a clean tube.
6. Centrifuge at 37,000 g for 40 minutes at 4°C.
7. Discard the supernatant.
8. Resuspend the pellet (P2) in half the original volume (volume added in step 2) with Extraction Buffer.

Extraction Buffer

Reagent	Concentration
Tris (pH 9)	50 mM
NaCl	150 mM
NP-40	1%
Sodium deoxycholate	0.5%
Phenylmethylsulfonyl fluoride (PMSF)	1 mM
Complete EDTA-free protease inhibitor cocktail (Roche)	

9. Incubate at 37°C for 30 minutes.
10. Centrifuge the solution at 100,000 x g for 60 minutes.
11. Transfer the resulting supernatant (enriched brain synaptosome) to a clean tube.
12. Determine protein concentration using the Bradford method and adjust to 4 mg/ml with Extraction Buffer.
13. Store samples at -80°C until further use.