SAMPLE PREPARATION FOR WESTERN BLOTTING

Choosing a Lysis Buffer

Most starting materials need to be lysed through the use of chemical or mechanical meth ods to release the protein(s) of interest so that they can migrate individually through a se parating gel. This can be done by taking into consideration the type of the sample, the su bcellular location of the protein of interest and the required conditions that will allow the antibody to recognize its epitope.

In most cases, however, researchers use chemical lysis buffers to disrupt the cellular mem branes and solubilize the target proteins. Some of the most commonly used lysis buffers i nclude Tris-HCl (for soluble cytoplasmic proteins), Tris-Triton (for cytoskeleton-bound cyt oplasmic proteins), and NP-40, RIPA buffer and Triton X-100 (for whole cell lysates and nu clear, mitochondrial and membrane-bound proteins). Buffers containing SDS and other io nic detergents are known to be extremely harsh so you can get the highest yield by using these.

However, please note that not all lysis buffers would recognize a protein that has been ex tracted with denaturing detergents such as SDS and deoxycholate. If your chosen lysis bu ffer does not recognize denatured proteins, it is best to use buffers that contain mildly io nic detergents or those that do not contain any harsh detergents instead. In such cases, c ell lysis is accomplished through mechanical shearing using a Dounce homogenizer or by passing cells through a syringe tip.

Adding Protease and Phosphatase Inhibitors

Once the cells rupture, your sample runs the risk of undergoing proteolysis so you may n eed to keep your samples on ice or under 4oC at all times. You may also need to add the appropriate protease or phosphatase inhibitor to preserve the integrity of your sample.

Some of the most commonly used protease inhibitors include aprotinin (inhibits the actio n of trypsin, chymotrypsin and plasmin), EDTA/EGTA (for metalloproteases that require m agnesium, manganese and calcium), Leupeptin (for lysosomal proteases), Pepstatin-A (for aspartic proteases) and PMSF (inhibits serine and cysteine). On the other hand, sodium fl uoride (inhibits acidic phosphatases), sodium orthovanadate (inhibits tyrosine and alkalin e phosphatases), B-glycerophosphate and sodium pyrophosphate (both inhibit serine and threonine phosphatases) are among the most commonly used phosphatase inhibitors.

Preparing the Lysate

The lysate can be prepared from a cell culture or directly from the tissue of interest. In bo

th cases, ice cold lysis buffer should be added to the starting material before agitating an d centrifuging them at 4℃. Cell cultures should be agitated for 30 minutes while tissue sa mples should be agitated for 2 hours prior to centrifuging them at 12000 rpm for 20 min utes. After preparing the lysate, the protein concentration should then be determined us ing the Bradford, Lowry or BCA assay. With everything else done, you are now ready to lo ad the lysate into the gel or store it at -20℃or -80℃ for future use.

Choice of loading buffer: The final step of sample preparation for western blotting is addi ng loading buffer to the protein sample and heating or no heating of sample depending upon the type of PAGE. For SDS-PAGE, loading buffer contains SDS, 2-mercaptoethanol, glycerol and bromophenol blue. SDS denature the protein and make them negatively cha rged, 2-mercaptoethanol or DTT break disulfide bonds and glycerol increases sample den sity so that proteins settle in the well when loading. Bromophenol blue is added to monit or the run of peptide/protein on gel. Usually for SDS-PAGE, sample is heated with loading buffer before loading on wells.

The loading buffer for native-PAGE does not contain SDS, 2-mercaptoethanol or DTT and sample are not heated with loading buffer before loading on gel.