Protein Ladder

1. What gel running buffer should be used: Tris-glycine, Tris-tricine, or Tris-acetate?

Most of the common gel running buffers are composed of Tris-glycine or Tris-tricine. Tris-glycine buffer systems are useful for the separation of proteins over a wide range of molecular weights (5-300 kDa) and are compatible with denaturing or non-denaturing conditions. The Tris-tricine buffer is generally recommended for the electrophoresis of low molecular weight proteins and peptides (<10 kDa) that need to be reduced and denatured prior to loading. The Tris-acetate buffer system is used for the separation of larger proteins (>200 kDa).

2. Why is the non-specific binding detected after Western blot?

Protein ladder bands can sometimes be detected with chemiluminescent techniques due to non-specific interactions of ladder proteins with either primary or secondary antibodies (or with both). The ladder bands are only rarely detected by chromogenic substrates. The extremely high sensitivity of the chemiluminescent assays is needed to see the bands, so the actual degree of cross-reactivity is low. The non-specific cross-reactivity is difficult to predict as it often has a different pattern depending on the antibodies used. If antibodies recognize a linear epitope, the cross-reactivity may be due to sequence homology. If antibodies react with a denaturation-resistant conformational epitope, it could be impossible to identify the exact reason for the detected cross-reactivity. The most general way to handle this problem would be to use lower concentrations of antibodies.

3. What is the precise concentration of the proteins in GeneDirex's prestained Ladders?

We do not provide precise protein concentrations in the Certificate of Analysis. We recommend that researchers should not do in-gel quantification; they should instead perform a BSA dilution series. If a researcher insists on doing in-gel quantification, it must be done with an unstained ladder.

4. Why do the apparent molecular weight values for the prestained protein ladders appear to be different in different gel types?

When a protein is covalently bound to a charge-carrying dye molecule, this can affect the protein's overall charge. Altering the protein's charge will most likely change its mobility within the gel. This explains why the prestained protein ladders are given "apparent" molecular weight values, while regular unstained protein ladders are given their true molecular weights. The apparent molecular weights stated on the data cards and other references of our prestained protein ladders were determined using Tris-Glycine, Tris-tricine, or Bis-Tris gels. The apparent molecular weight may seem "incorrect". The reason for this disparity is the different formulations of the gel types (buffering agents, ionic strength, and pH).

5. Why does few colors of protein marker become weak after washing ?

Most of time it is not happened. However, few strong detergent might cause red and green color reduction due to relative lower binding strength of these two colors by washing with strong detergent several times.

As following, we suggest the formulation of wash buffer and the stripping buffer:

Wash buffer

10mM phosphate buffer, pH 7.4,

2.7mM KCl,

140 mM NaCl,

0.05% Tween 20,

Stirpping buffer

0.2 M Glycine, pH2.8,

0.5M NaCl

6. We observe degradation of protein marker after electrophoresis. Why ?

Every batch of protein marker has passed strict QC before shipping. Degradation might be caused by unappreciated storage condition. We suggest to aliquot PM to avoid frequent freeze-thaw and store at -20°C. Another major possibility is contaminated by protease in environment. We suggest you to sanitize by ethanol before starting experiment, ware glove and do not reuse tips.