User Manual

Protein Medley™ Protocol

I. Introduction

Tissue-Specific Protein Concentrates are electrophoresis-ready solutions of SDS-solubilized proteins prepared from normal human tissues. The proteins are isolated from whole tissue homogenates under conditions designed to minimize proteolysis and to ensure maximal representation of tissue-specific proteins. All preparative steps are performed at 4°C, and protease inhibitors are present throughout the isolation procedure. Each tube contains a solution of total protein in sample buffer consisting of 62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 0.1% bromophenol blue (pH 6.8) (1). This protein solution is suitable for any application compatible with such detergent solubilized proteins. Refer to the Product Analysis Certificate (PAC) that accompanied your order for the concentration of your Protein Medley.

II. Sample preparation for one dimensional SDS-polyacrylamide gel electrophoresis (PAGE)

The following procedure is based on the discontinuous polyacrylamide gel system described by Laemmli (1). If an alternate electrophoresis system, or other application is employed, the sample preparation should be modified accordingly.

A. General considerations

1. It is not advisable to repeatedly freeze and thaw the protein concentrate. Therefore, if small amounts of the concentrate will be used at a given time we recommend that the concentrate be aliquoted at smaller volumes and refrozen at –20°C.
2. Thawing of the protein concentrate may yield a white precipitate which is due to the SDS coming out of solution. Gently warm the tube between your fingers until a clear blue solution is observed, then place the tube on ice for further manipulations.
3. Electrophoresis grade chemicals should be used for the preparation of all solutions.
4. The amount of total protein loaded per lane must be optimized for the particular application. Typical amounts of protein loaded on a mini-gel apparatus are in the range of 25-75 µg/lane for satisfactory separation of a protein mixture derived from a whole tissue homogenate.

B. Additional materials required

Note: The following materials are required but not supplied.

- Sample buffer (pH 6.8)

62.5 mM Tris	0.76	g
2% (w/v) SDS	2.0	g
10% (v/v) glycerol	10	ml
0.1% (w/v) bromophenol blue	0.1	g

Add Tris, SDS, and glycerol to 75 ml of ddH_2O.
Adjust pH to 6.8 with dilute HCl.
Add the bromophenol blue, and adjust to final volume of 100 ml with ddH_2O. Store buffer at room temperature.

- 1.0 M dithiothreitol

Add 1.54 g of dithiothreitol to 8 ml of ddH_2O, mix gently until dissolved.
Adjust final volume to 10 ml with ddH_2O.
Aliquot into microfuge tubes and freeze at –20°C. Stable for 1 month.

C. Preparation of protein sample for gel electrophoresis

1. Remove protein concentrate from freezer and allow to thaw at room temperature. Mix gently until a clear blue solution is observed, then place tube on ice.
2. Dilute the protein concentrate to the desired final concentration using sample buffer, or other suitable buffer for your application. Dilutions are best performed in 1.5 ml microfuge tubes or similar size vessel.

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3. For applications requiring the reduction of disulfide bonds (e.g. SDS-polyacrylamide reducing gels), add 1.0 M dithiothreitol to achieve a final concentration of 50 mM (this is a 20-fold dilution of the above stock solution).
4. Boil the diluted sample for 5 minutes by placing tube in a H_2O bath, or heat block.
5. Spin tubes for 10 minutes. Note: Avoid pellet when loading the solution into the gel.
6. Load samples on the gel. The final volume of sample should be approximately 50% of the available space in the gel lane.

III. Recommended application protocols

1. SDS-polyacrylamide gel electrophoresis (1, 2, 3)
2. Two-dimensional polyacrylamide gel electrophoresis (4)
3. Isoelectric focusing (5)
4. Western blotting (2, 3, 6, 7)
5. Immunoassays (2, 3)

IV. References

1. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-685.
2. Harlow, E. & Lane, E. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).
3. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).
4. O'Farrell, P. H. (1970) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**:4007-4111.
5. Scopes, R. K. (1987) *Protein Purification: Principles and Practice*, second edition (Springer-Verlag Inc., New York).
6. Towbin, H., Staehelin, T. & Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350-4356.
7. Bjerrum, O. J. & Heegaard, N. H. H. (eds.) (1988) *CRC Handbook of Immunoblotting of Proteins*, CRC Press Inc., Boca Raton, Florida.