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Product Information

Kit for Molecular Weights 14,000–500,000
Non-denaturing PAGE

Catalog Number **MWND500**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Non-denaturing (native) polyacrylamide gel electrophoresis is a sensitive technique for characterization of proteins. The electrophoretic mobility is dependent on the charge, shape (conformation), and mass of the native protein. Electrophoresis in non-denaturing systems allows the retention of these protein characteristics. Denaturing electrophoresis systems (urea and sodium dodecyl sulfate) typically mask the native protein charge and cause dissociation of proteins into subunits, leading to loss of most biological activity.

Non-denaturing electrophoresis systems can be used to study changes in protein charge, conformation, and mass. The charge on a native protein is pH dependent, making buffer selection important. If the pH of the buffer is near the isoelectric point (pI) of the protein, little migration into gel will be observed. Charge isomers may be observed for a protein due to post translational modifications, which add small charged moieties, or degradation (deamidation). Conformational changes may be due to differences in protein folding or binding of small ligands to the protein.

Observed changes in molecular mass may be due to formation of protein aggregates or oligimers, or an indication of protein:protein or protein:ligand binding. The markers in this kit may be used for estimation of molecular mass in non-denaturing electrophoresis systems. Determination of the molecular mass of an unknown protein requires running at least 3 gels of different polyacrylamide concentrations and use of Ferguson plots.[1,2] Procedures for characterizing molecular mass isomers and charge isomers in non-denaturing electrophoresis systems have been published.[2,3]

In non-denaturing systems proteins often retain their biological activity allowing in-gel detection using specific substrate stains and recovery of the active protein.

The proteins supplied in this kit have a molecular mass range of 14.2–545 kDa (see Table 1). α-Lactalbumin is characterized by a single band. Carbonic Anhydrase is characterized by three bands and Albumin from chicken egg white by two bands (charge isomers). Bovine Serum Albumin exhibits monomer and dimer bands and Urease has trimer and hexamer bands (molecular mass isomers).[4-6]

Components

The kit contains one vial of each lyophilized protein (1 mg of each). The 5 markers have a molecular mass range of 14.2–545 kDa (see Table 1).

Table 1.
MWND500 Individual Markers

Catalog Number	Protein (1 mg/vial)	Molecular Mass (kDa)
L4385	α-Lactalbumin from bovine milk	14.2
C5024	Carbonic Anhydrase from bovine erythrocytes	29
A7642	Albumin from chicken egg white	45
A8654	Albumin from bovine serum	66 (monomer) 132 (dimer)
U7752	Urease from Jack bean[6]	272 (trimer) 545 (hexamer)

Reagents Required but Not Provided

- Tris-Glycine Buffer 10× Concentrate, Catalog Number T4904
- Methanol, Catalog Number 179957
- Acetic Acid, Catalog Number A6283
- Brilliant Blue R Concentrate, Catalog Number B8647 **Or** EZBlue™ Gel Staining Reagent, Catalog Number G1041
- Glycerol, Catalog Number G5516
- 1 M Trizma® hydrochloride solution, pH 7.0 (Catalog Number T1819)
- Bromophenol Blue, Catalog Number B5525 **Or** 0.04% (w/v) Bromophenol Blue Solution (Catalog Number 318744)
- Apoferritin, Catalog Number A3641
  Although not included in this kit, Apoferritin, (molecular masses: monomer is 450 kDa; dimer is 900 kDa) is recommended for use as a marker when a higher range of molecular masses is required.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reagents

- Electrode Buffer – Dilute Tris-Glycine Buffer 10× Concentrate (Catalog Number T4904) 10-fold with water to yield 25 mM Tris with 192 mM glycine, pH ~8.3.

  Store tightly capped at 2–8 °C. Suitable for use in absence of visible microbial growth.

- Destaining Solution - Combine:
  400 ml methanol
  70 ml acetic acid
  530 ml water

- Staining Solution - The premixed EZBlue Gel Staining Reagent (Catalog Number G1041) may be used.
  Or
  Dilute Brilliant Blue R Concentrate (Catalog Number B8647) to 1 liter with water to yield 0.25% (w/v) Brilliant Blue R, 40% (v/v) methanol, and 7% (v/v) acetic acid.

  Store tightly capped at room temperature. This reagent is stable for several months.

- Tris Buffer – Dilute 1 M Trizma hydrochloride solution, pH 7.0 (Catalog Number T1819) 20-fold with water to yield 50 mM Tris Buffer.

- Sample Buffer – Combine one volume Tris Buffer, one volume glycerol (Catalog Number G5516), and one volume of 0.04% (w/v) Bromophenol Blue Solution (Catalog Number 318744).

Electrophoresis Gels

Slab gels may be prepared according to published procedures[7,8] or commercially available precast Tris-Glycine (Laemmli) gels may be used.

Molecular Mass Markers

Reconstitute the contents of each vial of protein marker with 1 ml of water, with the exception of Urease, Catalog Number U7752. Reconstitute the vial of Urease with 0.5 ml of water and mix until the protein is in solution. Then add 0.5 ml of glycerol to the Urease solution to stabilize the protein during freeze thaw cycles. Solutions may be frozen at –20 °C for future use. It is recommended that repeated freezing and thawing be avoided. Stock solutions may be dispensed into working aliquots, frozen, and then discarded after 2–3 uses.

Protein Sample

The concentration of each unknown protein sample should be ~1 mg protein per ml in an appropriate buffer. Dilute the sample with an equal volume of Sample Buffer.

Storage/Stability

Store the kit at –20 °C.

Procedure

Electrophoresis

1. Immediately before use, thaw an aliquot of each prepared protein marker and dilute with an equal volume of Sample Buffer.

2. Load the Molecular Mass Marker Solutions and unknown protein samples on appropriate gels. See Table 2 for suggested amounts of protein to be loaded for each Molecular Mass Marker Solution and protein sample.

Table 2.
Protein Amounts to be Loaded.

Protein	Protein Amount (µg)
α-Lactalbumin from bovine milk	5
Carbonic Anhydrase from bovine erythrocytes	5
Albumin from chicken egg white	5
Albumin from bovine serum	2.5
Urease from Jack bean	5
Unknow Protein Sample	2–10

3. Add Electrode Buffer and perform the electrophoresis of the gels according to manufacturer's instructions.

4. Remove gels from the cassette.

Staining and Destaining of Gels

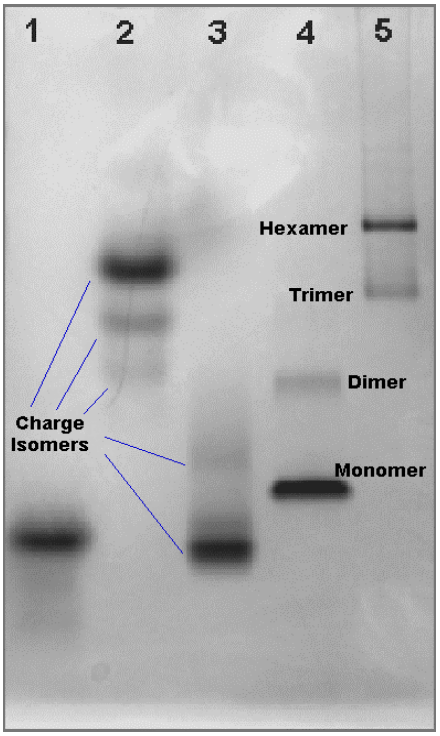
1. Stain gels in Staining Solution for ~1 hour.

2. No destaining is necessary if using EZBlue Gel Staining Reagent. Destain gels stained with Brilliant Blue R in Destaining Solution by diffusion against several changes of Destaining Solution.

3. Transfer the gels into 7% (v/v) Acetic Acid Solution for a minimum of 30 minutes to allow the gels to uniformly swell.

Results

Typical Electrophoretic Banding Patterns of Protein Markers (4-12% polyacrylamide gradient gel)



Lane 1 α-Lactalbumin
Lane 2 Carbonic Anhydrase
Lane 3 Albumin from chicken egg white
Lane 4 Albumin from bovine serum
Lane 5 Urease

References

1. Ferguson, K.A., Metabolism, **13**, 985 (1964).
2. Hedrick, J.L., and Smith, A.J., Arch. Biochem. Biophys., **126**, 155 (1968).
3. Chrambach, A., and Rodbard, D., Science, **172**, 440 (1971).
4. Fishbein, W.N., *et al*., Eur. J. Biochem., **73**, 185 (1977).
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6. Published amino acid sequence data for urease gives a subunit (monomer) molecular mass of 90,790 daltons,[9] and electron microscopy and molecular mass data suggest urease exists as trimeric and hexameric species.[4,5]
7. The Protein Protocols Handbook, 2nd ed., Walker, J.M., ed., Humana Press (Totowa, NJ: 2002), pp. 57-60.
8. Gel Electrophoresis of Proteins: A Practical Approach, 3rd ed., Hames, B.D., ed., IRL Press (Oxford, UK: 1998)
9. Mamiya, G., *et al*., Proc. Japan Acad., **61 (B)**, 09 (1985).

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