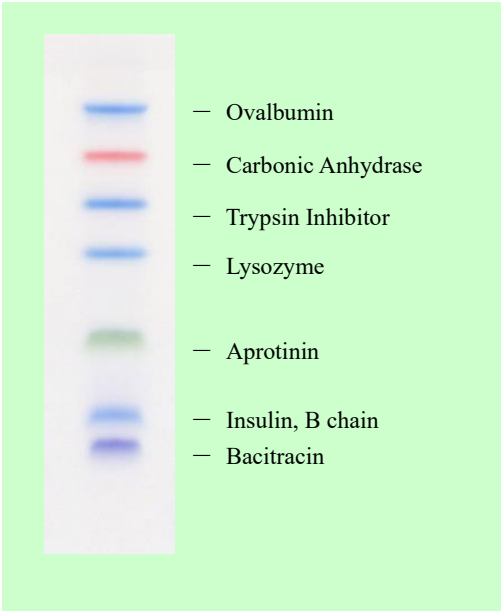
Product Name: DynaMarker Protein MultiColor Stable, Low Range

Code No:	DM670
Lot No:	002CA04
Size:	200 µl (40 mini-gel lanes)
Storage:	4 °C
Stability:	12 months at 4 °C
Storage Buffer:	50 mM Tris-HCl (pH6.8), Urea, SDS, Glycerol, EDTA

Description

DynaMarker Protein MultiColor Stable, Low Range is a pre-stained protein molecular weight marker. The marker is supplied in gel loading buffer for direct loading onto SDS-PAGE without heating or adding reducing agents. It is easy to start electrophoresis since the marker can be stored and stable at 4 °C in a liquid state. The marker consists of seven prestained protein standards, Blue, Red, Green and Purple, ranging in appropriate molecular weight from 2 kDa to 46 kDa. The marker is suitable for monitoring gel electrophoresis and electrophoretic transfer onto membranes. The protein concentrations are optimized to give uniform band intensities.



Electrophoresis profile of DynaMarker Protein MultiColor Stable, Low Range (5 µl) on 16% polyacrylamide (3 % C) Gel / Tris-Tricine-SDS as running buffer.

Protocol

1. Take the marker out of refrigerator.
2. Load 5 µl for mini-gels or more for large size gels.
3. Load your samples.
4. Start electrophoresis.

Note: There is no need to heat or add reducing agents.

BioDynamics Laboratory Inc.
www.biodynamics.co.jp

Contents

Protein	Color	Apparent molecular weight (kDa) *
Ovalbumin	Blue	46.3
Carbonic Anhydrase	Red	30.8
Trypsin Inhibitor	Blue	22.6
Lysozyme	Blue	17.0
Aprotinin	Green	8.7
Insline, B chain	Blue	3.9
Bacitracin	Purple	1.7

Running buffer : Tris-Tricine-SDS buffer [1]

Note: As covalently bound dye affects protein mobility, each batch of our prestained protein marker is calibrated against unstained standards. A prestained protein marker should be used for approximate molecular weight determination. For precise molecular weight determination, you should use an unstained molecular weight marker.

* **:** The apparent molecular weight values are lot specific.

Recommended usage

DynaMarker Protein MultiColor Stable, Low Range is suitable for monitoring low molecular weight protein on polyacrylamide gel electrophoresis using Tris-Tricine-SDS as running buffer [1], and for monitoring electrophoretic transfer.

One example is shown below:

・Detection of β-Amyloid (1-42)

1) Electrophoresis

1-1) Preparation of reagents [2]

・Acrylamide-Bis stock solution (T:49.5%, C:3%)

Acrylamide	48 g
Bis-acrylamide	1.5 g
dH_2O	to 100 mL

・3× Gel buffer

3 M Tris-HCl (pH8.45), 0.3 % SDS

・10× Anode buffer

1.0 M Tris-HCl (pH8.9)

・10×Cathode buffer

1.0 M Tris, 1.0 M Tricine, 1.0 M SDS (pH adjustment is not necessary)

・4×Sample buffer

150 mM Tris-HCl (pH7.0), 12 % SDS, 6 % mercaptoethanol, 30 % Glycerol, 0.05 % CBB

1-2) Preparation of 16 % polyacrylamide (3 % C) Gel

Prepare the 16 % separating gel solution according to the following table and pour the gel solution into the mold of a vertical gel apparatus. The separating gel is overlaid with several drops of water and left until polymerized adequately, and then the overlaid water was replaced by a 4 % stacking gel.

	4 % stacking gel	16 % separating gel
Acrylamid-Bis stock solution	1 ml	10 ml
3× Gel buffer	3 ml	10 ml
Glycerol	–	2.4 ml
dH_2O	8 ml	7.6 ml
10 % APS	90 µl	100 µl
TEMED	9 µl	10 µl

1-3) Sample preparation

Prepare the protein sample according to the following table.

Protein sample	15 µl
4× Sample buffer	5 µl
	20 µl

Heat at 95°C for 5 min, and then put the tube on ice.

1-4) Electrophoresis of the protein samples and DynaMarker® Protein MultiColor Stable, Low Range.

Set the Polymerized acrylamide gel in the electrophoresis apparatus, then pour the 1× Anode buffer and 1× Cathode buffer. Load the protein sample and 5 µl of DynaMarker® MultiColor Stable, Low Range into a well and run the gel at 100─200 V.

2) Transfer onto membrane

2-1) Preparation of reagents

・Transfer buffer (Towbin buffer)

25 mM Tris, 192 mM Glycine, 20 % MeOH

・Nitrocellulose membrane (0.2 µm pore size).

Note: Transfer efficiency differs depending on the type of membranes and buffers, because the physical properties of small molecule proteins greatly vary depending on kinds of proteins.

2-2) Semi-dry transfer

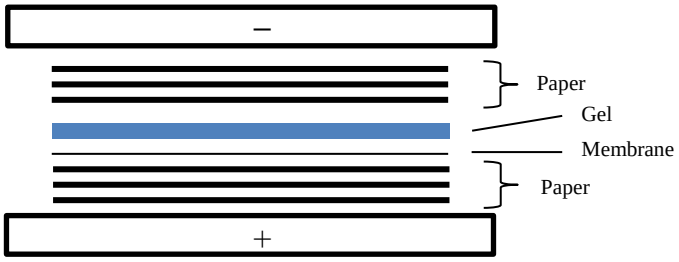


Figure 1, Semi-dry transfer

2-2-1) Prepare six sheets of blotting paper and a sheet of nitrocellulose membrane.

2-2-2) Soak the blotting paper and nitrocellulose membrane with transfer buffer for 10 min.

2-2-3) Place the three sheets of blotting paper on the anode platform of the transfer cell.

2-2-4) Place the membrane on top of the blotting paper.

2-2-5) Transfer the gel from the glass plate to the top of the membrane and press out any air bubbles.
(*It is important to make sure that there are no air bubbles between the membrane and the gel.)

2-2-6) Place the other three sheets of blotting paper onto the gel and set the cathode assembly.

2-2-7) Transfer for 60 min at 2 mA/cm^2.

2-2-8) After ensuring the marker has transferred successfully onto the membrane, remove the membrane from apparatus.

2-2-9) Rinse the membrane in PBS buffer.

3) Detection

3-1) Boil the membrane for a few minutes (3～5 min.) in PBS buffer [(3)]

3-2) Block with TBS based protein free blocking agent (e.g. Pierce® Protein-Free T20 (TBS) Blocking Buffer*) for 1 hr

3-3) Wash 3 times for 5 min in TBS-T(0.05% Tween20)

3-4) Incubate with anti β-Amyloid antibody (mouse monoclonal) overnight at 4 °C

3-5) Wash 3 times for 5 min in TBS-T(0.05% Tween20)

3-6) Incubate with biotinylated anti mouse IgG antibody for 1 hr at room temperature.

3-7) Wash 3 times for 5 min in TBS-T(0.05% Tween20)

3-8) Incubate with VECTASTAIN Elite ABC Standard kit**
(Please refer to the manual.)

3-9) Wash 3 times for 5 min in TBS-T(0.05% Tween20)

3-10) Stain with DAB Peroxidase substrate kit***.
(Please refer to the manual.)

*: Pierce Protein-Free T20 (TBS) Blocking Buffer is a product of Thermo Fisher scientific, Inc.

**: VECTASTAIN Elite ABC Standard kit is a product of Vector laboratories, Inc.

***: DAB peroxidase substrate kit is a product of Vector laboratories, Inc.

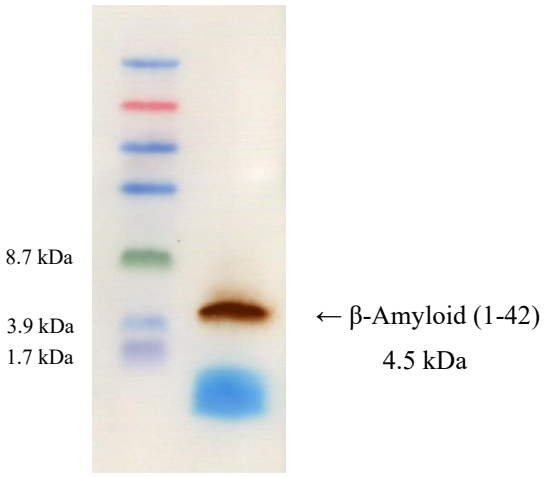


Figure 2, Detection of β-Amyloid (1-42)

Reference

(1) Hermann schägger and Gebhard Von Jagow. Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis for the Separation of Proteins in the Range from 1 to 100 kDa. *Anal. Biochem.* 166, 368-379 (1987).

(2) Hermann schägger. Tricine-SDS-PAGE. *Nature Protocols.* 1, 16-22 (2006)

(3) Nobuo Ida, *et.al*. Analysis of Heterogeneous βA4 Peptide in Human Cerebrospinal Fluid and Blood by a Newly Developed Sensitive Western Blot Assay. *J. Biol. Chem.* 271, 22908-22914 (1996)