GE Healthcare

Amersham Low Molecular Weight Calibration Kit for SDS Electrophoresis

A lyophilized mixture of six highly purified well-characterized proteins for use in molecular weight determination in the presence of sodium dodecyl sulphate (SDS)

Product Booklet

Code: 17-0446-01

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA
UK

2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only.*

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

The kit should be stored at 2–8°C.

2.3. Expiry

For expiry details see outer packaging.

3. Components

Protein mixture 576 µg/vial, 10 vials, each containing the following proteins:

Phophorylase b (1), rabbit muscle, 67 µg, molecular weight (M_r) 97 000

Albumin (2), bovine serum, 83 µg, M_r 66 000

Ovalbumin (3), chicken egg white, 147 µg, M_r 45 000

Carbonic anhydrase (4), bovine erythrocyte, 83 µg, M_r 30 000

Trypsin inhibitor (5), soybean, 80 µg, M_r 20 100

α-Lactalbumin (6), bovine milk, 116 µg, M_r 14 400

The amount of each protein has been chosen to give bands of equal intensity when stained with Coomassie™ Brilliant Blue following Laemmli-type gel electrophoresis. Intensities may vary when using other staining methods.

4. Other materials required

- Electrophoresis reagents appropriate to the application being run
- Detection reagents appropriate to the application being run
- Gel electrophoresis equipment

5. Description

The Low Molecular Weight SDS Calibration Kit for SDS electrophoresis is a lyophilized mixture of six highly purified well-characterized proteins for use in molecular weight estimation in the presence of the detergent sodium dodecyl sulphate (SDS).
The molecular mass of the protein under investigation is determined by comparing its electrophoretic mobility with that of proteins contained in the kit.

Ten vials are supplied, each containing a lyophilized mixture of highly purified protein standards of molecular mass range (M_r) 14 400 to 97 000 when used in denaturing polyacrylamide electrophoresis.

6. Protocol

6.1. Preparation of calibration kit

Preparation of the calibration proteins depends on the detection method used, as described below. When reconstituted as directed, the six calibrated proteins will be in a 25% sucrose solution, so it is not necessary to add a density enhancing agent. For best reproducibility, discard any unused portion of the reconstituted protein solution. However, if necessary, the solution can be stored at -80°C for 3 months.

For Coomassie Brilliant Blue detection

For Laemmli gels (Figure 2), reconstitute the contents of a vial in 200 µl of a standard 1x sample buffer [0.0625 M Tris-HCl, 2% SDS, 10% (v/v) glycerol (optional), 0.1 M DTT and 0.01% bromophenol blue, pH 6.8].

For PhastGel™, ExcelGel™ and CleanGel™ precast gels, reconstitute the contents of a vial in 200 µl of 10 mM Tris-HCl, 2% SDS, 0.1 M DTT, 0.01% bromophenol blue and 1 mM EDTA, pH 8.0.

For silver stain detection

For silver staining (Figure 3), reconstitute the contents of a vial as described for Coomassie blue staining, then dilute aliquots by at least 50-fold in 1x sample buffer.

6.2. Denaturing proteins

Heat the reconstituted protein solution for 5 minutes at 95–100°C.

6.3. Gel loading

Select the appropriate sample volume from the table:

Gel type	Sample volume (µl)
Vertical mini	1–8
Vertical standard	2–8
Multiphor™ II flatbed	1–3
PhastSystem™	0.3–4

6.4. Electrophoresis

Perform electrophoresis according to the instructions supplied with the gel apparatus being used.

6.5. Detection

Stain the gel using the desired method.

6.6. Molecular weight determination

Measure the migration distance of the proteins in the Calibration Kit and of the protein(s) of interest. Measure the migration distance of the dye marker. Calculate the corresponding R_f values by dividing migration distance of the protein by migration distance of the dye marker.

Construct a calibration curve by graphing R_f vs. log molecular weight for the proteins in the Calibration Kit (Figure 1). Determine the molecular weight of the protein(s) of interest from the calibration curve.

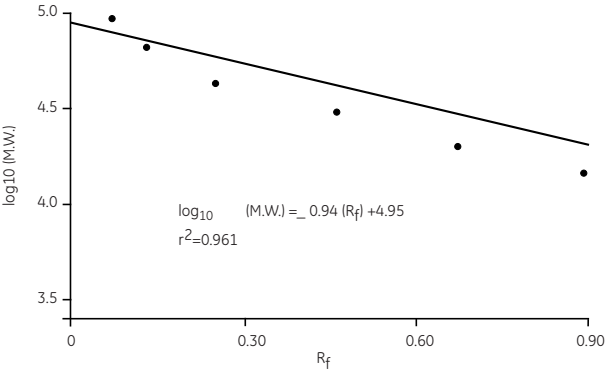

Fig 1. Calibration curve constructed using results shown in figure 2.

7. Typical results

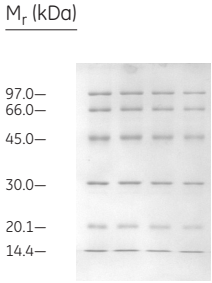

Fig 2. LMW standards stained with Coomassie Brilliant Blue. Aliquots (3 µl per lane) of a 2 x dilution were loaded on a self-cast 15% T, 2.7% C gel. The gel was run at a constant current of 20 mA for 1 hour, 55 minutes on a Mighty Small™ electrophoresis unit. The gel was stained with PhastGel Blue R (17-0518-01).

Protein	M_r(Da)	R_f
Phophorylase b	97 000	0.07
Albumin	66 000	0.13
Ovalbumin	45 000	0.25
Carbonic anhydrase	30 000	0.46
Trypsin inhibitor	20 100	0.67
α-Lactalbumin	14 400	0.89

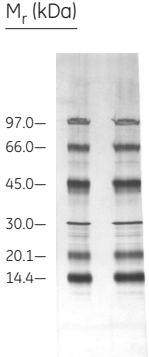

Fig 3. LMW standards stained with silver stain. Aliquots (5 µl) of a 50 x dilution were loaded per lane on an ExcelGel SDS gradient 8-18 (80-1255-53), run at 600 V, 50 mA for 80 minutes on Multiphor II flatbed unit. The gel was stained with PlusOne™ Silver Staining Kit, Protein (17-1150-01) using a Hoefer™ Automated Gel Stainer

Protein	M_r(Da)	R_f
Phophorylase b	97 000	0.16
Albumin	66 000	0.26
Ovalbumin	45 000	0.41
Carbonic anhydrase	30 000	0.57
Trypsin inhibitor	20 100	0.71
α-Lactalbumin	14 400	0.80

8. Additional Information

8.1. Background and references

For further information regarding molecular weight determinations and denaturing electrophoresis, see Hoefer Protein Electrophoresis Applications Guide (80-6013-88)

1. Titani, K. *et al., Proc. Natl. Acad. Sci. USA* **74**, 4762–4766 (1977).

2. Hirayama, K. *et al., Biochem. Biophys. Res. Comm.* **173**, 639–646 (1990).

3. Yao, J. *et al., Anal. Chem.* **67**, 3638–3642 (1995).

4. Reynaud, J. *et al., Biochimie.* **53**, 1095–1098 (1971).

5. Koide, T. and Ikenaka, T., *Eur. J. Biochem.* **32**, 401–407 (1973).

6. Brew, K. *et al., J. Biol. Chem.* **242**, 3747–3749 (1967).

8.2. Related products

PhastGel Blue R (40 Coomassie Blue R-350 tablets)	17-0518-01
PlusOne silver Staining Kit, protein	17-1150-01
Hoefer Automated Gel Stainer with 19 x 29 cm PTFE coated staining tray with 29 x 35 cm PTFE coated staining tray	 80-6395-02 80-6396-16
Hoefer Protein Electrophoresis Application Guide	80-6013-88

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84
Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111
Freiburg
Germany

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway
NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1
Hyakunincho Shinjuku-ku
Tokyo 169-0073
Japan

GE Healthcare regional office contact numbers:

Asia Pacific
Tel: +85 65 62751830
Fax: +85 65 62751829

Australasia
Tel: + 61 2 8820 8299
Fax: +61 2 8820 8200

Austria
Tel: 01/57606-1613
Fax: 01/57606-1614

Belgium
Tel: 0800 73 890
Fax: 02 416 8206

Canada
Tel: 1 800 463 5800
Fax: 1 800 567 1008

Central, East, & South East Europe
Tel: +43 1 972 720
Fax: +43 1 972 722 750

Denmark
Tel: 45 70 25 24 50
Fax: 45 45 16 2424

Eire
Tel: 1 800 709992
Fax: +44 1494 542010

Finland & Baltics
Tel: +358 9 512 3940
Fax: +358 9 512 39439

France
Tel: 01 69 35 67 00
Fax: 01 69 41 98 77

Germany
Tel: 0800 9080 711
Fax: 0800 9080 712

Greater China
Tel: +852 2100 6300
Fax: +852 2100 6338

Italy
Tel: 02 26001 320
Fax: 02 26001 399

Japan
Tel: +81 3 5331 9336
Fax: +81 3 5331 9370

Korea
Tel: 82 2 6201 3700
Fax: 82 2 6201 3803

Latin America
Tel: +55 11 3933 7300
Fax: + 55 11 3933 7304

Middle East & Africa
Tel: +30 210 96 00 687
Fax: +30 210 96 00 693

Netherlands
Tel: 0800-82 82 82 1
Fax: 0800-82 82 82 4

Norway
Tel: +47 815 65 777
Fax: +47 815 65 666

Portugal
Tel: 21 417 7035
Fax: 21 417 3184

Russia, C.I.S. & N.I.S
Tel: +7 495 956 5177
Fax: +7 495 956 5176

Spain
Tel: 902 11 72 65
Fax: 935 94 49 65

Sweden
Tel: 018 612 1900
Fax: 018 612 1910

Switzerland
Tel: 0848 8028 10
Fax: 0848 8028 11

UK
Tel: 0800 515 313
Fax: 0800 616 927

USA
Tel: +1 800 526 3593
Fax: +1 877 295 8102

http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
UK

Imagination at work

17-0446-01PL Rev D 2006