

GE Healthcare

Amersham High Molecular Weight Calibration Kit for native electrophoresis

A lyophilized mixture of five highly purified well-characterized proteins for use in molecular weight estimation under non-denaturing conditions.

Product Booklet

Code: 17-0445-01



Page finder

1. Legal	3
2. Handling	4
2.1. Safety warnings and precautions	4
2.2. Storage	4
2.3. Expiry	4
3. Components	5
4. Other materials required	6
5. Critical parameters	7
6. Description	8
7. Protocol	9
7.1. Preparation of calibration kit	9
7.2. Gel loading	9
7.3. Electrophoresis	9
7.4. Detection	10
7.5. Molecular weight determination	10
8. Typical results	11
8.1. Electrophoresis gel samples of HMW Calibration kit for native electrophoresis	11
9. Background and references	13
10. Related products	14

1. Legal

GE and GE monogram are trademarks of General Electric Company. Amersham, Hoefer, Multiphor, PlusOne, PhastGel and PhastSystem are trademarks of GE Healthcare companies.

Coomassie is a trademark of Imperial Chemical Industries Ltd

© 2006 General Electric Company – All rights reserved.

GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specification and features shown herein, or discontinue the product described at any time without notice or obligation.

Contact your GE Healthcare representative for the most current information and a copy of the terms and conditions.

<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 250 µg/vial, 10 vials contains the following proteins:

Thyroglobulin (1), porcine thyroid, 76 µg, molecular weight (M_r) 669 000
Ferritin (2), equine spleen, 50 µg, M_r 440 000
Catalase (3), bovine liver, 36 µg, M_r 232 000
Lactate dehydrogenase (4), bovine heart, 48 µg, M_r 140 000.

Albumin (5), bovine serum, 40 µg, M_r 66 000.

The amount of each protein has been chosen to give bands of equal intensity when stained with Coomassie™ Brilliant Blue following 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. Critical parameters

- Reconstitute the HMW standard vial in appropriate buffer.
- Not recommended for use in denaturing systems i.e. containing sodium dodecyl sulphate.

6. Description

The High Molecular Weight Calibration Kit is a lyophilized mixture of five highly purified well-characterized proteins for use in molecular weight estimation under non-denaturing conditions.

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) 66 000 to 669 000.

7. Protocol

7.1. Preparation of calibration kit

Reconstitute the contents of a vial in **100 μ l** of the electrophoresis sample buffer appropriate to the application being run. When reconstituted in this volume, the protein solution will also contain about 25% sucrose. It is therefore not necessary to add sucrose, glycerol or other density enhancing agents to the sample buffer.

For Coomassie Brilliant Blue detection

Load reconstituted standards without further dilution.

For silver stain detection

Dilute the reconstituted proteins by at least 50-fold in the electrophoresis sample buffer appropriate to the application being run.

7.2. Gel loading

Select the appropriate sample volume from the table:

Gel type	Sample volume (μl)
Vertical mini	5–10
Vertical standard	10–20
Multiphor™II flatbed	5–20
PhastSystem™	0.3–4

7.3. Electrophoresis

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

7.4. Detection

Stain the gel using the desired method.

7.5. Molecular weight determination

The electrophoretic mobility of non-denatured proteins depend on their charge and shape as well as their molecular mass. It is therefore generally not possible to estimate the molecular mass of a non-denaturing protein using the HMW Calibration Kit on a single gel. The molecular mass of a non-denaturing protein can be estimated by running multiple gels of different polyacrylamide concentrations and plotting R_f vs. acrylamide concentration for each standard (Ferguson plots (6,7)).

Note: These standards are not recommended for use in denaturing gel electrophoresis in systems containing sodium dodecyl sulphate (SDS, Laemmli gels). Some of the standards consist of multiple subunits and will dissociate under denaturing conditions. Dissociation may not be complete. further complicating interpretation.

8. Typical results

8.1. Electrophoresis gel samples of HMW Calibration Kit for native electrophoresis

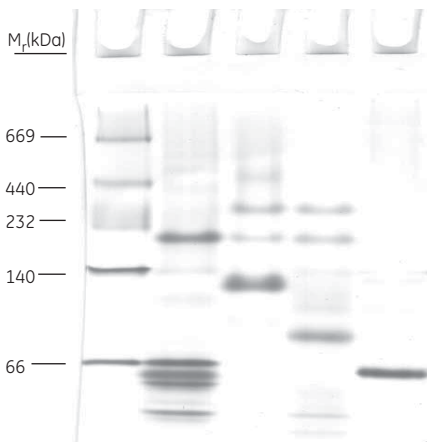


Fig 1. HMW Calibration Kit proteins and various protein samples run on a 5–12.5% polyacrylamide gradient gel. 10 μ l of a two-fold dilution of the HMW calibration proteins (leftmost lane) and various protein samples were run on a 1 mm thick 5–12.5% gradient gel.

Electrophoresis buffer system: The Tris-Glycine, Tris-Chloride discontinuous system of Ornstein and Davis was used (8) (see also the Hoefer™ Protein Electrophoresis Applications Guide).

Electrophoresis conditions: the gel was run at 15 mA constant current on a Hoefer SE260 mini-vertical apparatus until the bromophenol blue dye front exited the gel.

Staining: The gel was stained with Coomassie™ Blue R350.

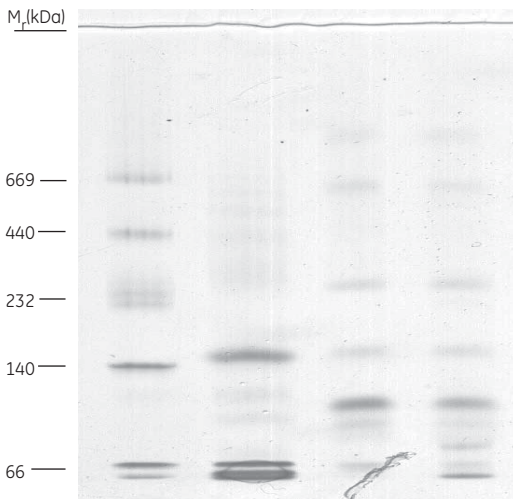


Fig 2. HMW Calibration Kit proteins and various protein samples run on a 4–15% PhastGel™ gradient using native buffer strips. 1 μ l of a two-fold dilution of the HMW calibration proteins (leftmost lane) and various protein samples were run on a 4–15% PhastGel gradient using native buffer strips. The 8/1 μ l sample applicator was used.

Electrophoresis conditions: The gel was run as described in PhastSystem Separation Technique File No. 130.

Staining: The gel was stained with Coomassie Blue R350 as described in PhastSystem Development Technique File No. 200.

9. Background and references

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

1. Spiro, M. J., *J. Biol. Chem.* **248**, 4446-4460, (1973).
2. Bryce, C. F. A. and Crichton, R. R., *J. Biol. Chem.* **246**, 4198-4205, (1971).
3. Takeda, A. *et al.*, *J. Biochem.* **78**, 911-924, (1975).
4. Huston, S. J. *et al.*, *Biochem.* **11**, pp.1609-1612, 1972.
5. Hirayama, K. *et al.*, *Biochem. Biophys. Res. Comm.* **173**, 639-646, (1990).
6. Gallagher, S. R., Native Discontinuous Electrophoresis and Generation of Molecular Weight Standard Curves (Ferguson Plots). In *Current Protocols in Protein Science* (Coligan, J. E. *et al.* eds.) p. 10.3.5, John Wiley & Sons, New York, (1995).
7. Andrews, A. T., *Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications*, 2nd ed. Oxford University Press, New York, (1986).
8. Davis, B. J., *Ann. N.Y. Acad. Sci.* **121**, 404, (1964).

10. 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	80-6395-02
with 29 x 35 cm PTFE coated staining tray	80-6396-16
Hoefer Protein Electrophoresis Applications 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 6 275 1830
Fax: +85 65 6 275 1829

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 82 06

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

**Central, East, & South
East Europe**
Tel: +43 1 972720
Fax: +43 1 97272 2750

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

Eire
Tel: 1 800 709992
Fax: 0044 1494 542010

Finland & Baltics
Tel: +358-(0)9-512 39 40
Fax: +358 (0)9 512 39 439

France
Tel: 01 6935 6700
Fax: 01 6941 9677

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 9600 687
Fax: +30 210 9600 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 & other 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