Amersham High Molecular Weight Calibration Kit for SDS Electrophoresis

Product Booklet

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17061501PL AC

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1 Introduction

Product code

17061501

About

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

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Human blood products provided as components of this pack have been obtained from donors who were tested individually and were found to be negaive for the presence of Human Immunodeficency Virus antibody (HIV-Ab)[1] as well as for Hepatitis B surface Antigen (HBsAg) using approved methods (ELISA)

[1] HIV is the abbreviation used for HTLV-III and LAV.

As no test method can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus antibody (HIV-Ab)[1] or other infectious agents are absent, all human blood products should be considered potentially infectious. Handling, use, storage and disposal should be in accordance with the procedures defined by an appropriate National biohazard safety guideline or regulation, where it exists (for example USA Centre for Disease Control/National Institutes of Health manual "Biosafety in microbiological and Biomedical Laboratories", 2nd Edition 1988).

Storage

The kit should be stored at 2–8°C.

Expiry

For expiry details see outer packaging.

Components

Protein mixture

176 µg/vial, 10 vials, each containing the following proteins:

Myosin (1), rabbit muscle, 25 µg, molecular weight (M_r) 220000

α_2-Macroglobulin (2), bovine plasma, 100 µg, M_r 170000

β-Galactosidase (3), *E.coli*, 16 µg, M_r 116000

Transferrin (4), human, 17 µg, M_r 76000

Glutamic dehydrogenase (5), bovine liver, 18 µg, M_r 53000

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.

2 Other materials required

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

3 Description

The High Molecular Weight SDS Calibration Kit for SDS electrophoresis is a lyophilized mixture of five highly purified wellcharacterized 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) 53000 to 220000 when used in denaturing polyacrylamide electrophoresis.

4 Protocol

Preparation of calibration kit

Reconstituting the contents of one vial in 100 µL of water gives a protein solution in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 33 mM KCl, 2.5% dithiothreitol (DTT) and 2.5% SDS. The solution also contains 12% mannitol (stabilizer and density enhancer) and xylene cyanol green (tracking dye). It is not

necessary to heat the calibration kit components in order to denature them before use. 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

Aliquots from the reconstituted solution can be applied directly to the gel of choice for Coomassie Blue staining (*Fig. 2, on page 8*). However, if further dilutions are desired, use a standard 1x sample buffer (0.0625 M Tris-HCl, 2% SDS, 10% v/v glycerol, 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 100 µL of 10 mM Tris-HCl, 2% SDS, 0.1 M DTT, 0.01% bromophenol blue and 1mM EDTA, pH 8.0.

For silver stain detection

For silver staining (*Fig. 3, on page 9*), reconstitute the contents of a vial as described for Coomassie blue staining, then dilute aliquots by at least 50-fold in 1 x sample buffer.

Gel loading

Select the appropriate sample volume from the table:

Gel type	sample volume (µL)
Vertical mini	3–10
Vertical standard	3–10
Multiphor™ II flatbed	2–4
PhastSystem™	0.3–4

Electrophoresis

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

Detection

Stain the gel using the desired method.

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 (*Fig. 1, on page 7*). Determine the molecular weight of the protein(s) of interest from the calibration curve.

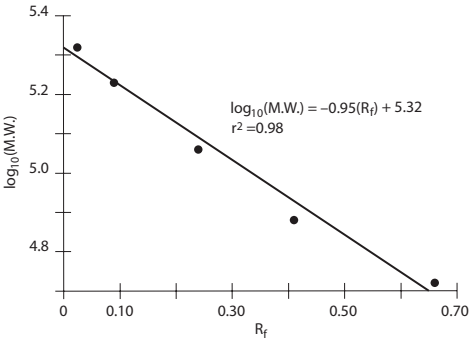

Fig 1. Calibration curve constructed using results shown in *Fig. 2, on page 8*.

5 Typical results

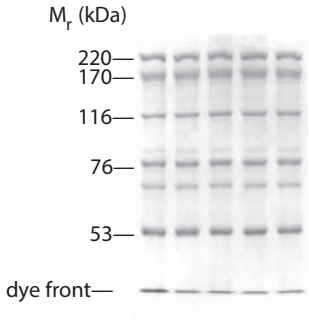

Fig 2. HMW-SDS standards stained with Coomassie Brilliant Blue.

Aliquots (10 µL per lane) of a 2 x dilution were loaded on a self-cast 7.5% T, 2.7% C gel. The gel was run at a constant current of 20 mA for 1 hour, 42 minutes on a Mighty Small electrophoresis unit. The gel was stained with PhastGel Blue R (17-0518-01).

Protein	M_r(Da)	R_f
Myosin	220 000	0.02
α_2-Macroglobulin	170 000	0.09
β-Galactosidase	116 000	0.24
Transferrin	76 000	0.41
Glutamic dehydrogenase	53 000	0.66

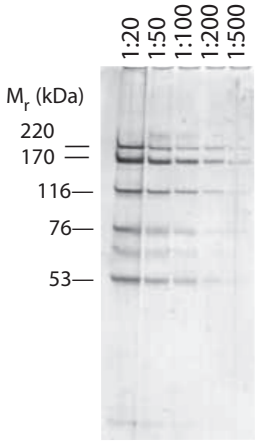

Fig 3. HMW -SDS standards stained with silver stain.

Aliquots (5 µL per lane) of a dilution series were loaded on an ExcelGel SDS Homogeneous 12.5(80-1261-01), run at 600 V, 50 mA, 30 W for 80 minutes on a Multiphor II flatbed unit. The gel was stained with PlusOne Silver Staining Kit, Protein (17-1150-01). The dilution factor, with respect to reconstitution of a vial in 100 µL, is indicated in each lane.

Protein	M_r(Da)	R_f
Myosin	220 000	0.08
α_2-Macroglobulin	170 000	0.12
β-Galactosidase	116 000	0.19
Transferrin	76 000	0.28

Protein	M_r(Da)	R_f
Glutamic dehydrogenase	53 000	0.40

6 Related products

PhastGel Blue R (40 Coomassie Blue R-350 tablets)	17051801
PlusOne silver Staining Kit, protein	17115001
Hoefer Automated Gel Stainer with 19 x 29 cm PTFE coated staining tray	80639502
with 29 x 35 cm PTFE coated staining tray	80639616
Hoefer Protein Electrophoresis Application Guide	80601388

7 Background and references

For further information regarding molecular weight determinations and denaturing electrophoresis, see Hoefer Protein Electrophoresis Applications Guide (80601388)

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3. Fowler, A. V. and Zabin, I., Proc. Natl. Acad. Sci. USA. 74, 1507- 1510 (1977)
4. Roberts, R. C. et al., J. Biol. Chem. **241**, 4907-4913 (1966).
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