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
1. Legal

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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.) 669 000
Ferritin (2), equine spleen, 50 μg, M, 440 000 Catalase (3), bovine
liver, 36 μg, M, 232 000 Lactate dehydrogenase (4), bovine heart,
48 μg, M, 140 000.
Albumin (5), bovine serum, 40 μg, M, 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) 66 000 to 669 000.
7. Protocol

7.1. Preparation of calibration kit
Reconstitute the contents of a vial in 100 μl 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:

<table>
<thead>
<tr>
<th>Gel type</th>
<th>Sample volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical mini</td>
<td>5–10</td>
</tr>
<tr>
<td>Vertical standard</td>
<td>10–20</td>
</tr>
<tr>
<td>Multiphor™II flatbed</td>
<td>5–20</td>
</tr>
<tr>
<td>PhastSystem™</td>
<td>0.3–4</td>
</tr>
</tbody>
</table>

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

![Figure 1](image1.png)

*Fig 1.* HMW Calibration Kit proteins and various protein samples run on a 5–12.5% polyacrylamide gradient gel. 10 μl 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 μl 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 μl 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).

10. Related products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Code</th>
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<tbody>
<tr>
<td>PhastGel Blue R 40 Coomassie Blue R-350 tablets</td>
<td>17-0518-01</td>
</tr>
<tr>
<td>PlusOne™ silver Staining Kit, protein</td>
<td>17-1150-01</td>
</tr>
<tr>
<td>Hoefer Automated Gel Stainer with 19 x 29 cm PTFE coated staining tray</td>
<td>80-6395-02</td>
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<tr>
<td>with 29 x 35 cm PTFE coated staining tray</td>
<td>80-6396-16</td>
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<tr>
<td>Hoefer Protein Electrophoresis Applications Guide</td>
<td>80-6013-88</td>
</tr>
</tbody>
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