



IEF MARKERS

Product Nos. **A 2910, G 7146, T 1021, L 5137, C 3666, C 6403, C 6653, M 9267, L 1277, T 1146, M 5768**

Product Description

The use of individual protein markers enables the investigator to select a marker mixture best suited for the pH range being studied. Thus, the needless waste of proteins which would stack on the anode or cathode is eliminated. In pI determinations, individual protein markers should be used in conjunction with at least two or three other markers as part of a standard curve.

Storage

Store lyophilized form below 0 °C with desiccant. Stable for 12 months at -20 °C after reconstitution.

Preparation Instructions

Reconstitute each vial as follows:

Prod No.	Weight (mg)	Reconstitution Volume (ml)	Concentration (mg/ml)
A 2910	2	0.50	4
G 7146	2	0.50	4
T 1021	2	0.50	4
L 5137	2	0.50	4
C 6403	2	0.50	4
C 6653	2	0.50	4
M 9267	2	0.50	4
T 1146	2	0.50	4
L 1277	1	0.25	4
C 3666	1	0.25	4
M 5768	---	1.0	---

Each vial contains glycine at a concentration of 200 mM at a reconstituted protein concentration of 4 mg/ml.

Solutions may be diluted for use as individual markers or combined for use as pI standards in the desired pH ranges. In either case, the recommended final protein concentration should be 0.4-0.6 mg/ml per marker except for Product No. L 1277 which should have a final concentration of 0.8-2.0 mg/ml. Product No. M 5768 (Methyl Red) should not be diluted further.

Product Information

Note: Lentil Lectin (Product No. L 1277) and β -Lactoglobulin A (Product No. L 5137) will be hazy upon reconstitution. This in no way affects the electrofocusing, and results in an insignificant loss of protein.

Procedure

Application of solutions to the gel may be accomplished in several ways. Small paper applicators are available which will apply approximately 15 μ l. Protein samples, usually up to 10 μ l in volume, may also be applied directly to the gel as drops, streaks, or rectangles. Basins may be made in the gel into which the sample is introduced; however, the depth of the basin should be limited to less than 30% of the gel thickness. Deeper troughs may skew the pH gradient.¹

One mm thick gels (5% acrylamide and 3% cross-linking agent) should be run at 1 W/cm gel length. If paper applicators are used, they should be removed 30 minutes into a run. One additional hour should be sufficient time to complete the electrofocusing. If a constant power supply is not available, run at 25 V/cm for 30 minutes and 50 V/cm for 5 1/2 hours.

For further information on electrofocusing and Sigma isoelectric point markers, see Sigma Technical Bulletin No. IEF-100A.

Reference

1. Righetti, P. G. and Drysdale, J. W., Isoelectric Focusing Laboratory Techniques in Biochemistry and Molecular Biology (Work, T.S. and Work, E., gen. eds.) North Holland Publishing Co., Amsterdam, New York, Oxford (1976)

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