# Instructions for use

## ROTIPHORESE®-PAGE Matrix Buffer plus

8996

**4x concentrated, for electrophoresis**

### Gel matrix buffer for enhanced protein separation and significantly higher gel strength.

ROTIPHORESE®-PAGE Matrix Buffer plus is a 4x concentrated neutral pH buffer. It can be used for preparation of both stacking and resolving gel *by simply replacing the Laemmli/TRIS-, or TRICIN-, buffer system*.

### Gel properties:

- Even gels with stacking gels of only one acrylamide concentration show a *protein separation quality comparable to that of gradient gels*, when prepared with ROTIPHORESE®-PAGE Matrix Buffer plus. Band depiction is significantly enhanced compared to standard buffer systems.
- The neutral pH buffer improves the stability of gel resulting in *longer shelf lives* of self-made gels.
- ROTIPHORESE®-PAGE Matrix Buffer plus provides *increased gel strength*, making handling of the gels much more convenient, for instance during separation of the glass plates or during transport. Even a 6% wide range gel offer the same gel strength as a 10% Laemmli gel.

**Figure 1:** 6 % PA gel

### Compatibility:

Gels prepared with ROTIPHORESE®-PAGE Matrix Buffer plus can be used

- with all common sample and running buffers
- for all standard staining methods including CBB and silver staining
- for gel extraction, ligation, transformation and transfection
- for 2D protein analysis or subsequent Western Blotting onto NC or PVDF membrane

### Separation range:

Gradient gels offer a much wider separation range of proteins than single percentage gels. However, casting gradient gels is more difficult and labor intensive.

ROTIPHORESE®-PAGE Matrix Buffer offers a gradient gel-like separation on a single percentage gel. Gels prepared with ROTIPHORESE®-PAGE Matrix Buffer plus provide a much greater separation range than gels casted with a conventional Laemmli buffer system. The band dissemination ranges from 5 kDa to 250 kDa.

### Preparation of Gel Solutions:

ROTIPHORESE®-PAGE Matrix Buffer plus replaces the Laemmli/TRIS-, or TRICIN-, buffer system. The buffer does not contain SDS.

**Separation Gel (unit: ml)**

using **40 %** Acrylamid-/Bisacrylamide solution

| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
|---|---|---|---|---|---|
| 6 % | Water | 5.9 | 11.8 | 17.7 | 23.6 |
| | ROTIPHORESE® Gel 40 (37,5:1) | 1.5 | 3 | 4.5 | 6 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.008 | 0.016 | 0.024 | 0.032 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 8 % | Water | 5.4 | 10.8 | 16.2 | 21.6 |
| | ROTIPHORESE® Gel 40 (37,5:1) | 2 | 4 | 6 | 8 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 10 % | Water | 4.9 | 9.8 | 14.7 | 19.6 |
| | ROTIPHORESE® Gel 40 (37,5:1) | 2.5 | 5 | 7.5 | 10 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 12 % | Water | 4.4 | 8.8 | 13.2 | 17.6 |
| | ROTIPHORESE® Gel 40 (37,5:1) | 3 | 6 | 9 | 12 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |

using **30 %** Acrylamid-/Bisacrylamide solution

| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
|---|---|---|---|---|---|
| 6 % | Water | 5.4 | 10.8 | 16.2 | 21.6 |
| | ROTIPHORESE® Gel 30 (37,5:1) | 2 | 4 | 6 | 8 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.008 | 0.016 | 0.024 | 0.032 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 8 % | Water | 4.7 | 9.5 | 14.2 | 18.9 |
| | ROTIPHORESE® Gel 30 (37,5:1) | 2.7 | 5.3 | 8 | 10.7 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 10 % | Water | 4.1 | 8.1 | 12.2 | 16.3 |
| | ROTIPHORESE® Gel 30 (37,5:1) | 3.3 | 6.7 | 10 | 13.3 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |
| Acrylamide | Reagent | 10 ml | 20 ml | 30 ml | 40 ml |
| 12 % | Water | 3.4 | 6.8 | 10.2 | 13.6 |
| | ROTIPHORESE® Gel 30 (37,5:1) | 4 | 8 | 12 | 16 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 2.5 | 5 | 7.5 | 10 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.1 | 0.2 | 0.3 | 0.4 |
| | TEMED | 0.006 | 0.012 | 0.018 | 0.024 |

* prepare freshly!

Be careful to mix the solution thoroughly before and after addition of TEMED. Avoid bubbles. Pour gel immediately and overlay with isopropanol.

**Stacking Gel (unit: ml)**

using **40 %** Acrylamid-/Bisacrylamide solution

| Acrylamide | Reagent | 2 ml | 4 ml | 6 ml | 8 ml |
|---|---|---|---|---|---|
| 3 % | Water | 1.33 | 2.66 | 3.99 | 5.32 |
| | ROTIPHORESE® Gel 40 (37,5:1) | 0.15 | 0.3 | 0.45 | 0.6 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 0.5 | 1 | 1.5 | 2 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.02 | 0.04 | 0.06 | 0.08 |
| | TEMED | 0.002 | 0.004 | 0.006 | 0.008 |

using **30 %** Acrylamid-/Bisacrylamide solution

| Acrylamide | Reagent | 2 ml | 4 ml | 6 ml | 8 ml |
|---|---|---|---|---|---|
| 3 % | Water | 1.28 | 2.56 | 3.84 | 5.12 |
| | ROTIPHORESE® Gel 30 (37,5:1) | 0.2 | 0.4 | 0.6 | 0.8 |
| | ROTIPHORESE®-PAGE Matrix Buffer plus | 0.5 | 1 | 1.5 | 2 |
| | 10 %-Ammonium Peroxodisulfate Solution | 0.02 | 0.04 | 0.06 | 0.08 |
| | TEMED | 0.002 | 0.004 | 0.006 | 0.008 |

* prepare freshly!

Be careful to mix the solution thoroughly before and after addition of TEMED.
Avoid bubbles. Pour the stacking gel immediately and insert the comb carefully.

The acrylamide/ bisacrylamide mixture ready-made solutions used in this tables are as follows:

- ROTIPHORESE® Gel 40 (29:1), Art. No. A515
- ROTIPHORESE® Gel 40 (37.5:1), Art. No. T802
- ROTIPHORESE® Gel 30 (37.5:1), Art. No. 3029

## Comparison of Separation:

Classical PAGE gel produced with Tris glycine SDS buffer versus PAGE gel produced with ROTIPHORESE®-PAGE Matrix Buffer plus:

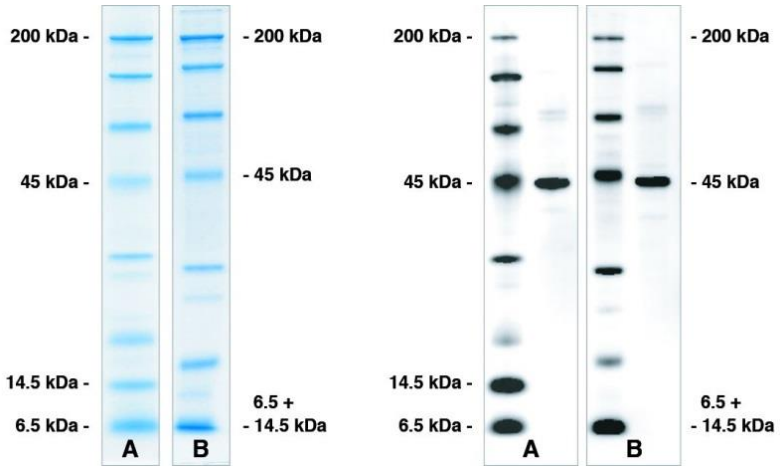



**Figure 2 and 3:**
Coomassie® stained gel and Western blot. HeLa extract on PVDF, detected via HRP chemoluminescence.
A: 10 % PAGE using ROTIPHORESE®-PAGE Matrix Buffer plus
B: 12 % PAGE using standard Tris glycine SDS buffer

The *results* show that the gel prepared with ROTIPHORESE®-PAGE Matrix Buffer plus provides superior separation of the close size small proteins (6.5 kDa and 14 kDa) compared to the conventional Laemmli gel. Besides, the resolutions of high molecular weight proteins (45-200 kDa) are wider than Laemmli gel.

## Detection of ß-Actin by Western Blotting

ROTIPHORESE®-PAGE Matrix Buffer plus can be used for Western blotting* with the standard detection protocol (fig. 3).

***Please note:** When transferring proteins that have been separated on gels containing ROTIPHORESE®-PAGE Matrix Buffer plus, these should ideally be blotted onto PVDF membranes and transferred with transfer buffer containing SDS (0.01 % - 0.1 %). When blotting small proteins onto nitrocellulose membranes, the transfer buffer should also include SDS. Optimise transfer conditions carefully to prevent small proteins from passing through the membrane. Please also refer to our Technical Info Brochure "Transfer Buffers and Parameters".

Gel A: 10 % PA in ROTIPHORESE®-PAGE Matrix Buffer plus
Gel B: 12 % PA in standard Laemmli buffer
Samples: Hela cell extract, blotted onto PVDF membrane (10 V, 30 mins.)
Primary Antibody: Mouse anti β-Actin, monoclonal (1:200, 60 mins.)
Secondary Antibody: Goat anti mouse IgG(H+L), HRP-conjugated (1:5.000)
Detection by chemoluminescence

## 2D Electrophoresis with Silver Staining

ROTIPHORESE®-PAGE Matrix Buffer plus is suitable for silver staining.

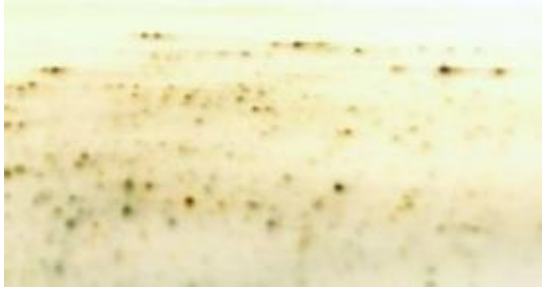
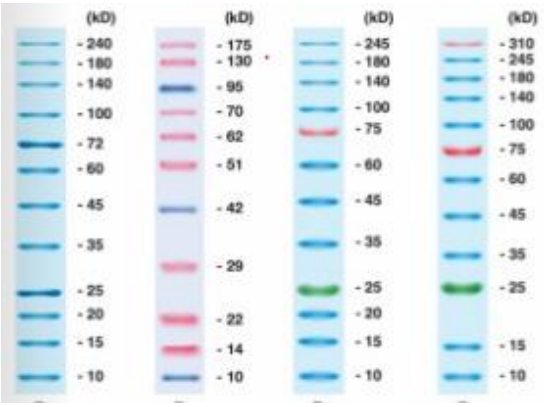


**Figure 4:**
HL-60 cell extract after 1D-IEF (pH 4-7) and 2D gel electrophoresis in a 12 % PA gel using ROTIPHORESE®-PAGE Matrix buffer plus.

## Recommended Products

| Art.No. | Product | Conc. | Ratio* |
|---|---|---|---|
| **Acrylamide/Bisacrylamide Mixtures, Ready-to-Use** | | | |
| 3029 | ROTIPHORESE® Gel 30 (37.5:1): | 30 % | 37.5:1 |
| 3030 | ROTIPHORESE® Gel 40 (19:1): | 40 % | 19:1 |
| A515 | ROTIPHORESE® Gel 40 (29:1): | 40 % | 29:1 |
| T802 | ROTIPHORESE® Gel 40 (37.5:1): | 40 % | 37.5:1 |
| **PAGE Reagents** | | | |
| 9592 | Ammonium peroxodisulphate (APS) | | |
| 2367 | N,N,N',N'-Tetramethylethylendiamine (TEMED) | | |
| 1057 | ROTI®-Stock 20 % SDS (solution) | 20 % | |
| 2326 | SDS Ultra-Pure (powder) | | |
| 8029 | SDS Pellets, ROTIPHORESE®-Grade, for Electrophoresis | | |
| **Alternative Running Buffer** | | | |
| 3060 | ROTIPHORESE® 10x SDS-PAGE | 10x | |
| 1249 | ROTI®Fair SDS-PAGE, premixed powder in pouches | 1000 ml / pouch | |
| 1250 | ROTI®Fair SDS-PAGE, premixed powder in pouches | 5000 ml / pouch | |
| **Gel loading buffers** | | | |
| K929 | ROTI®Load 1 (SDS) – with β-mercaptoethanol | 4x | |
| K930 | ROTI®Load 2 (SDS) – without β-mercaptoethanol | 4x | |
| 3359 | ROTI®Load 3 (LDS) – without β-mercaptoethanol | 4x | |
| **Protein Markers** | | | |
| 9299 | ROTI®Mark PETIT, 7 bands, 3.5-40 kDa, not prestained | | |
| 2242 | ROTI®Mark ALL BLUE, 12 bands, 10-240 kDa, **prestained** (1) | | |
| 8269 | ROTI®Mark BI-PINK, 11 bands, 10-175 kDa, **prestained** (2) | | |
| 8271 | ROTI®Mark TRICOLOR, 12 bands, 10-245 kDa, **prestained** (3) | | |
| 2244 | ROTI®Mark TRICOLOR XTRA, 12 bands, 10-310 kDa, **prestained** (4) | | |

* Acrylamide : Bisacrylamide




2242 (1) 8269 (2) 8271 (3) 2244 (4)

**Content:**
One bottle of 250 ml ready-made buffer solution is sufficient for pouring of approx. 100 mini gels.

**Transport and Storage:**
Store at +4 °C. Shipped on cool packs.

**Safety information:**
**Danger** H302-H315-H319-H317-H340-H350-H361f-H372
P201-P280-P301+P312-P302+P352-P305+P351+P338-P308+P313

## ROTIPHORESE®PAGE Matrix Buffer plus

**8996.1 plastic 250 ml**

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