

ZOOM® Strip pH 9-12

Cat. no. ZM0017

Store at -20°C

Introduction

The ZOOM® Strip pH 9-12 is a pre-cast immobilized pH gradient (IPG) gel cast on a plastic backing. ZOOM® Strips are easy to use and produce reproducible pH gradients.

ZOOM® Strips pH 9-12 offer a linear pH range of 9-12 that is ideal for isoelectric focusing (IEF) and analysis of basic proteins such as nucleic acid binding proteins, transcription factors, and ribosomal proteins.

Specifications

Length of the gel:	7.0 cm
Length of the ZOOM® Strip:	7.7 cm
Gel thickness:	0.5 mm
Width of the ZOOM® Strip:	3.3 mm
pH range:	9-12 linear
Storage:	Store at -20°C
Stability:	3 months at -20°C
Number of ZOOM® Strips	12/package

The anode (acidic) end is marked as (+) and cathode (basic) end as (-). Each ZOOM® Strip is individually numbered for easy identification.

Amount of Protein

ZOOM® Strips pH 9-12 are specifically formulated to allow increased protein loads. We recommend loading 50-100 µg (for silver staining) or 100-200 µg (for Coomassie® staining) of total protein per ZOOM® Strip pH 9-12.

Part No. ZM0017.pps

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This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Preparing Samples

To obtain the best results with ZOOM® Strips pH 9-12, use the sample preparation protocol described below. **It is important to perform reduction and alkylation prior to IEF to reduce horizontal streaking.**

1. Prepare 100X Protease Inhibitor Cocktail by dissolving 1 Protease Inhibitor Cocktail tablet (Roche cat. no. 1873580) in 500 μ l 1.1X ZOOM® 2D Protein Solubilizer 1 or 2. Mix well.
2. Prepare the Lysis Buffer **fresh**, just prior to use. You will need 950 μ l Lysis Buffer for each 1 ml of cell/tissue lysate.

1.1X ZOOM® 2D Protein Solubilizer 1 or 2 (cat. nos. ZS10001 or ZS10002)	909 μ l
1 M Tris Base	3 μ l
100X Protease Inhibitor Cocktail from Step 1	10 μ l
2 M DTT	10 μ l
Deionized water	18 μ l

Mix well and store on ice until use.

3. To 50 mg (wet weight) minced tissue or 50 μ l packed *E. coli* (1×10^{10}) cells, add 950 μ l chilled Lysis Buffer from Step 2.
4. Sonicate sample **on ice** for 5-10 rounds of 15 seconds each at ~50% power with cooling samples on ice between sonications.
5. Check the pH of the solution. The pH should be 8.4-9.0. Adjust the pH with 1 M Tris Base if needed.
6. Incubate on a rotary shaker for 10-15 minutes at room temperature.
7. Add 5 μ l 99% N,N-Dimethylacrylamide (DMA, from Aldrich cat. no. 27413-5) to the lysate for alkylation.
8. Incubate on a rotary shaker for 30 minutes at room temperature.
9. Add 10 μ l 2 M DTT to quench any excess DMA.
10. Centrifuge at 16,000 \times g for 20 minutes at 4°C. Use supernatant for IEF (next page) or aliquot into small volumes and store at -80°C.

Diluting Samples for IEF

Dilute the lysate from Step 10, previous page, for use with the ZOOM® IPGRunner™ System as described below. You will need 140 µl diluted sample per ZOOM® Strip.

Lysate from Step 10, previous page	9 µl
1.1X ZOOM® 2D Protein Solubilizer 1 or 2	128 µl
2 M DTT	1.4 µl
ZOOM® Carrier Ampholytes, pH 9-11 (cat. no. ZM0024)	0.4 µl
Trace Bromophenol Blue	
Deionized water	to 140 µl

Rehydrating ZOOM® Strips

ZOOM® Strips are supplied dry and are rehydrated in the ZOOM® IPGRunner™ Cassette (cat. no. ZM0003). For detailed instructions on rehydration and assembling the ZOOM® IPGRunner™ Mini-Cell (cat. no. ZM0001), see the ZOOM® IPGRunner™ System Manual (available from our Web site at www.invitrogen.com).

1. After diluting your sample as described above, load 140 µl of the diluted sample into each Enclosed Channel of the ZOOM® IPGRunner™ Cassette. Leave unused channels empty.
2. Peel a ZOOM® Strip pH 9-12 away from the card backing and slide the acidic end (+) of the strip into the cassette channel until the acidic end of each strip touches the end of the channel slot.
3. Seal all Sample Loading Wells (including unused wells) with Sealing Tape (supplied with ZOOM® IPGRunner™ Cassettes).
4. Incubate ZOOM® Strips in the ZOOM® IPGRunner™ Cassette for **1 hour** at room temperature. Then assemble the ZOOM® IPGRunner™ Mini-Cell (see ZOOM® IPGRunner™ Manual) and perform isoelectric focusing (see page 4).

Run Conditions for ZOOM® Strips pH 9-12

To obtain the best results, we recommend using ZOOM® Strips pH 9-12 with the ZOOM® IPGRunner™ Mini-Cell (cat. no. ZM0001).

1. After rehydrating ZOOM® Strips, assemble the ZOOM® IPGRunner™ Mini-Cell as described in the ZOOM® IPGRunner™ Manual.
2. Perform IEF using an appropriate protocol as described below:

Voltage Ramp	Step Voltage
175 V for 15 minutes	200 V for 20 minutes
175-2000 V ramp for 45 minutes	450 V for 15 minutes
2000 V for 1 hour	750 V for 15 minutes
	2000 V for 60 minutes

3. After the run is complete, disassemble the ZOOM® IPGRunner™ Mini-Cell as described in the ZOOM® IPGRunner™ Manual.

Product Qualification

ZOOM® Strips pH 9-12 are qualified by subjecting a mixture of proteins to isoelectric focusing under standard focusing conditions. The strips are stained and visualized for proper resolution and migration of protein bands.

Limited Use Label License

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