IEF pH 3-10 Buffer Kit, Mini

1. Catalog No. KIB010H

2. Kit Contents

Description	Quantity	Storage
Anode Buffer (50X)	50 ml	Store at room temperature
Cathode Buffer (pH 3-10) (10X)	100 ml	Store at 4°C
IEF Sample Buffer (2X)	15 ml	Store at 4°C

3. Protocol

1. Prepare your sample by adding one part sample to one part IEF Sample Buffer (2X) and mix well. Typically, 10-20mM salt concentration is optimum for isoelectric focusing. In some cases, a higher salt concentration is required for protein solubility, however, this may interfere with isoelectric focusing.
2. Dilute the IEF Cathode Buffer (10X) 1:9 with deionized water before use and de-gas the IEF Cathode Buffer (1X working solutions) for 10 minutes under vacuum, or purge 1 minute with nitrogen or helium gas just before using. This reduces the possibility of bubbles from dissolved carbon dioxide forming during the gel run. Fill the upper buffer chamber with the appropriate amount of Cathode Buffer.
3. Dilute the IEF Anode Buffer (50X) 1:49 with deionized water before use and pour the appropriate amount of Anode Buffer into the lower buffer chamber
4. Load appropriate volume of sample into the wells which have been filled with IEF Cathode Buffer.
5. Run the gel according to the following running conditions.

Voltage	125 V constant – 1 hour 200 V constant – 1 hour 500 V constant – 30 minutes
Approx. Current	Start 6mA / 1.0mm gel End 2mA / 1.0mm gel
Approx. Run Time	2.5 hours

Turn off the power when the dye is migrated to the end of the gel.

6. After the run, remove the gel from the cassette and fix the gel with fixing solution (12% TCA with 3.5% Sulfosalicylic Acid) for 30 minutes. This step is important to fix the proteins and to remove the ampholytes. Otherwise, a high background may result. After fixing, wash the gel 3 times with D.I. water.
7. Place the gel in stain (0.1% Coomassie R-250) and shake for 1 hr. Destain with a 1X solution of destain until the desired clarity has been achieved. All fixing, staining and destaining should be done with gentle shaking.

4. Buffer composition

Anode Buffer (50x)	
Phosphoric Acid	350 mM
D.W.	Up to 50 ml

Cathode Buffer (10x)	
Arginine (free base)	200 mM
Lysine (free base)	200 mM
D.W.	Up to 100 ml

IEF Sample Buffer (pH 3-10) (2x)	
Arginine (free base)	40 mM
Lysine (free base)	40 mM
Glycerol	30%
D.W.	Up to 15 ml