RotoLyte Ranges	(A) Acidic Component (B) Basic Component	pH Gradient (80%A):(20%B)	pH Gradient (50%A):(50%B)	pH Gradient (20%A):(80%B)	Catalog Number
2.9-4.1	(A) Propionic Acid				
	(B) DL-Serine	2.9-3.7	3.2-3.9	3.4-4.1	170-3026
3.9-5.6	(A) MES				
	(B) GLY-GLY	3.9-4.7	4.5-5.0	4.8-5.6	170-3027
4.5-6.1	(A) MOPSO				
	(B) ß-Alanine	4.5-5.7	5.2-5.7	5.4-6.1	170-3028
4.9-6.2	(A) MOPS				
	(B) γ-Amino-n-Butyric Acid	4.9-5.6	5.4-6.0	5.8-6.2	170-3029
5.1-6.8	(A) TAPS				
	(B) ε-Amino-n-Caproic Acid	5.5-6.3	5.7-6.4	6.2-6.8	170-3030
6.0-7.2	(A) HEPPS				
	(B) Creatinine	6.0-6.5	6.4-7.0	6.6-7.2	170-3031
6.4-7.5	(A) CAPSO				
	(B) ε-Amino-n-Caproic Acid	6.4-7.1	6.7-7.2	7.2-7.5	170-3032
6.9-8.2	(A) AMPSO				
	(B) ß-Picoline	6.9-7.6	7.4-7.9	7.8-8.2	170-3033
7.2-8.6	(A) AMPSO				
	(B) Bis-Tris	7.2-8.1	7.8-8.3	8.2-8.6	170-3034
7.8-8.9	(A) Hydroxyproline				
	(B) Bis-Tris	7.8-8.4	8.3-8.7	8.5-8.9	170-3035
8.5-9.5	(A) ε-Amino-n-Caproic Acid				
	(B) Bis-Tris	8.5-9.0	8.8-9.3	9.0-9.5	170-3036
9.2-10.4	(A) ε-Amino-n-Caproic Acid				
	(B) Triethanolamine	9.2-9.8	9.6-10.1	9.8-10.4	170-3037
9.5-11.0	(A) ε-Amino-n-Caproic Acid				
	(B) Tris Base	9.5-10.1	9.6-10.6	9.9-11.0	170-3038

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RotoLyte Instruction Manual

Ampholyte-Free Preparative Electrofocusing Buffers for the Rotofor® Protein Purification System

BIO-RAD

Introduction

Analytical isoelectric focusing (IEF) and preparative IEF procedures historically use synthetic carrier ampholytes to generate pH gradients. However, ampholytes can interfere with some analyses and may not be bio-compatible with some protein samples. RotoLyte buffers address the need for non-ampholyte pH gradients in preparative IEF with the following advantages

- No interactions between RotoLyte buffers and proteins
- Protein fractions are easily freed of the low molecular weight RotoLyte buffers
- RotoLyte buffers are non-antigenic and biocompatible
- RotoLyte buffers do not interfere with protein determination assays
- RotoLyte buffers are compatible with all non-ionic protein solubilizing agents

RotoLyte buffers are designed specifically to be used with the Rotofor cell. RotoLyte buffers consist of 13 different buffer pairs with closely spaced pK_as. When voltage is applied to a solution of RotoLyte buffers, the buffer pairs generate shallow, linear pH gradients between their individual pK_as. The RotoLyte series of buffer pairs covers the pH range from 2.9 to 11.0. Adjustments of the ratios of the two components of the individual buffer pairs enable every pH in the range to be

achieved. Because of the very narrow pH gradients they generate, RotoLyte buffers can provide better resolution of proteins with closely-spaced pIs than can most ampholyte gradients.

Important Note

When using RotoLyte buffers, optimum resolution is obtained when the pI of the protein to be purified falls near the middle of the pH gradient. All other proteins, with pIs falling outside the gradient, are forced toward the anode or cathode and are isolated in the extreme end fractions of the Rotofor focusing cell's chamber. Therefore, the pI of the protein of interest must be known prior to selecting a specific RotoLyte buffer pair. Determining the pI of a protein of interest is best accomplished by conventional analytical IEF-PAGE, with Bio-Rad's Model 111 Mini IEF Cell (catalog number 170-2975).

It is advisable to verify a new pH gradient with a protein-free trial run before purification. Following focusing and fraction collection, the pH of each fraction can be measured with any standard pH meter.

Specifications

Unit volume (buffers A and B)	100 ml each
Stock concentration (buffers A and B)	200 mM per component
Storage	4 ˚C
Shelf life	One year

Instructions

Thirteen RotoLyte buffer pairs are available. Each set consists of two bottles, A and B. For each set of RotoLyte buffers, Bottle A contains the more acidic of the two components, while bottle B contains the more basic component. The pH gradients, or separation zones, are created by combining component A with component B. A customized pH gradient for a given application can be produced by mixing A and B in different proportions.

To produce the desired working RotoLyte solution, combine component A, component B and sample (or deionized water) in the following proportions. Recommended running time, when using RotoLyte buffers in the Rotofor cell, is 4 hours.

Mini Rotofor Chamber (20 ml)

	80%A:20%B	50%A:50%B	20%A:80%B
Buffer A	8 ml	5 ml	2 ml
Buffer B	2 ml	5 ml	8 ml
Water and Sample	10 ml	10 ml	10 ml

Standard Rotofor Chamber (60 ml)

	80%A:20%B	50%A:50%B	20%A:80%B
Buffer A	24 ml	15 ml	6 ml
Buffer B	6 ml	15 ml	24 ml
Water and Sample	30 ml	30 ml	30 ml

Refer to the Rofofor cell instruction manual for detailed operating procedures.

The table below indicates the various approximate pH values for different combinations of components A and B for each set of RotoLyte buffer.