Pharmalyte

Instructions for Use

Carrier ampholytes for isoelectric focusing

Pharmalyte™ carrier ampholytes are unique products entirely developed by Cytiva. The procedures and conditions described below give optimal results with Pharmalyte.

Working dilution

The buffering capacity of Pharmalyte is 0.35 mmol/pH/ml.

Pharmalyte should be used at a final dilution of 1:16 in polyacrylamide and agarose gels, 1:15 in Ultrodex gels and 1:40 in columns.

Gel preparation

It is recommended to cast all slab gels on plastic support films. For polyacrylamide gels use GelBond PAGfilm (Product code 80112936 or 80112937). Polyacrylamide gels can also be cast on glass plates treated with Bind-Silane (Product code 17133001). For agarose gels use GelBond PAGfilm (Product code 80112932 or 80112933).

Polyacrylamide gels (T=5%, C=3%)

For best results we recommend PlusOne Acrylamide IEF (Product code 17130001/02) or PlusOne ReadyMix IEF (Product code 17130901) suitable for isoelectric focusing.

For maximal ease-of-use we recommend solutions, PlusOne Acrylamide IEF 40% solution (Product code 17130101) or PlusOne ReadySol IEF T40C3 (Product code 17131001).

Shelf life

Pharmalyte products have a shelf life of 3 years from date of manufacture when stored according to recommendations.

For lots released in 2011 and later, the expiry date is specified on the product label. For lots manufactured prior to 2011, the expiry date can be determined as 3 years after the manufacturing date stated on the product label.

Preparative IEF in Ultrodex gels

Use Ultrodex granulated gel (Product code 80113001) for preparative purposes.

Focusing conditions

Isoelectric focusing in slab gels is best performed in the MultiPhor™ II electrophoresis and isoelectric focusing unit (Product code 18101806) with EPS 3501 XL (Product code 19350001).

Table 1 and *Table 2* give the recommended electrode solutions for the different gel types and intervals. Always use power limited conditions. Sometimes current must also be limited. *Table 3, on page 2* and *Table 4, on page 3* give the recommended focusing conditions.

Electrode solutions

Table 1. Recommended electrode solutions for polyacrylamide slab gels

Pharmalyte pH interval	Anode (+) solution	Cathode (-) solution
2.5 to 5	0.1 M H_2SO_4	0.2 M L-histidine or 0.1 M NaOH
4 to 6.5	0.04 M (DL) glutamic acid	0.2 M L-histidine
4.2 to 4.9	0.04 M (DL) glutamic acid	0.1 M NaOH
4.5 to 5.4	0.04 M (DL) glutamic acid	0.1 M NaOH
5 to 6	0.04 M (DL) glutamic acid	0.1 M NaOH
5 to 8	0.04 M (DL) glutamic acid	1 M NaOH[1]
6.7 to 7.7	0.04 M (DL) glutamic acid	0.1 M NaOH
8 to 10.5	0.25 M HEPES	1 M NaOH
3 to 10	0.04 M (DL) aspartic acid	1 M NaOH

[1] Substitute 0.1 M NaOH when working with 230 mm gradients

Table 2. Recommended electrode solutions for Ultrodex gels

Pharmalyte pH interval	Anode (+) solution	Cathode (-) solution
2.5 to 5	0.1 M H_3PO_4	0.1 M HEPES
4 to 6.5	Pharmalyte 4–6.5 or 0.1 M H_3PO_4	Pharmalyte 4–6.5 or 0.1 M NaOH
5 to 8	Pharmalyte 5–8 or 0.1 M H_3POs_4	Pharmalyte 5–8 or 0.1 M NaOH
8 to 10.5	0.1 M HEPES	0.1 M ethylenediamine

Table 3. Recommended focusing conditions for using Pharmalyte in polyacrylamide gels (0.5 mm thick gels, inter-electrode distance of 100 mm, gel length of around 200 mm[1, 2])

Pharmalyte pH interval		Voltage (V)	Current (mA)	Power (W)	Time (h)	Approx. volthours (Vh)	Running temp. (°C)
2.5 to 5		2000	150	15 to 20	1.5 to 2	3000 to 4000	10
4 to 6.5		2000	150	15	1.5 to 2	3000 to 4000	10
	or	3000	150	25 to 30	1 to 1.5	3000 to 4000	10
5 to 8		2000	150	15	1.5 to 2	3000 to 4000	10
	or	3000	150	25 to 30	1 to 1.5	3000 to 4000	10
8 to 10.5		2000	150	15 to 20	1.5 to 2	3000 to 4000	10
4.2 to 4.9	[3]	3000	150	5	0.5	500	10
	[4]	3000	150	15	2.5	4000	10
4.5 to 5.4	[3]	3000	150	5	0.5	500	10
	[4]	3000	150	15	2	3500	10
5 to 6	[3]	3000	150	5	0.5	500	10
	[4]	3000	150	15	2.5	4000	10
6.7 to 7.7	[3]	3000	50	10	0.4	5000	20[5]
	[4]	3000	50	10	1.5	2000	20
	then	3000	50	15	0.3	500	20
3 to 10		2000	150	15	1.5 to 2	3000 to 4000	10
	or	3000	150	25 to 30	1 to 1.5	3000 to 4000	10

[1] For ultrathin gels, reduce the power in proportion to the gel thickness.

[2] For an inter-electrode distance of 200 mm use: 3000 V, 150 mA, 10 W for 12 000 to 15 000 Vh (4 to 5.5 h).

[3] Prefocusing conditions. To obtain optimal results, gels must be prefocused. After prefocusing, samples are applied 1.5 to 2 cm from the cathode.

[4] Focusing conditions.

[5] Optimal temperature for hemoglobins. For other applications, use 10°C.

Table 4. Recommended focusing conditions for using Pharmalyte in Ultrodex gels

Pharmalyte pH interval	Voltage (V)	Current (mA)	Power (W)	Time (h)	Approx. volthours (Vh)
Analytical IEF (gel 100 × 200 × 1 or 2 mm). Inter-electrode distance 100 mm					
All intervals	2000	150	15 to 30	1.5 to 2	2000 to 3000
Analytical IEF (gel 100 × 200 × 1 or 2 mm). Inter-electrode distance 200 mm					
4 to 6.5	3000	150	40 to 60	4 to 6	8000 to 12 000
5 to 8	3000	150	40 to 60	4 to 6	8000 to 12 000
2.5 to 5	3000	150	20 to 30	4 to 7	8000 to 12 000
8 to 10.5	3000	150	20 to 30	4 to 7	8000 to 12 000
3 to 10	3000	150	25 to 30	4 to 5	7000 to 9000
Preparative IEF (gel 125 × 260 × 5 mm) Inter-electrode distance approx. 200 mm					
4 to 6.5	3000	150	20 to 30	5 to 8	8000 to 12 000
2.5 to 5	3000	150	20 to 30	5 to 8	8000 to 12 000
8 to 10.5	3000	150	10 to 15	6 to 9	8000 to 12 000
3 to 10	3000	150	10 to 15	6 to 9	8000 to 12 000

Staining

All standard fixing and staining procedures may be used with gels containing Pharmalyte.

Troubleshooting

To obtain optimal results, prefocusing is generally recommended.

The interference of atmospheric CO_2 when focusing in pH gradients above pH 6.5 can be minimized by placing wicks soaked in 1 M NaOH on the cooling plate at either side of the gel.

Should other specific problems arise you are always welcome to contact your local Cytiva representative.

Ordering information

Pharmalyte pH intervals	Pack size (ml)	Product code
2.5 to 5	25	17045101
4 to 6.5	25	17045201
5 to 8	25	17045301
8 to 10.5	25	17045501
4.2 to 4.9	25	17046201
4.5 to 5.4	25	17046301
5 to 6	25	17046401
6.7 to 7.7	25	17046601
3 to 10	25	17045601

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