SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Acrylamido Buffers for Isoelectric Focussing in Immobilized pH Gradients (IEF in IPGs)

IEF in IPGs is an electrophoretic technique in which the pH gradients are immobilized by making the buffering groups responsible for the pH gradient an integral part of a polyacrylamide gel matrix. By linear mixing of two solutions of acrylamido buffers narrow and wide gradients in the pH range from 2.5 to 11 can be created. When incorporeted into the gel, the acrylamido buffers provide even and controlled buffering capacity and a uniformly low conductivity throughout the gradient. The covalent binding of the gradient eliminates cathodic drift. Therefore it is possible to apply high field strength to ultra-narrow pH gradients and to achieve resolutions as high as 0.001 pH units.

Product range

Sigma-Aldrich offers the 10 acrylamido buffers as solids as well as 0.2M solutions. All solutions can also be purchased combined in a set.

Order number, name	Package
01711 Acrylamido buffer pK 1	1g/5g
01712 Acrylamido buffer solution pK 1	10 ml
01713 Acrylamino buffer pK 3.1	1g/5g
01714 Acrylamino buffer solution pK 3.1	10 ml
01715 Acrylamino buffer pK 3.6	1g/5g
01716 Acrylamino buffer solution pK 3.6	10 ml
01717 Acrylamino buffer pK 4.6	1g/5g
01718 Acrylamino buffer solution pK 4.6	10 ml
01719 Acrylamino buffer pK 6.2	1g/5g
01721 Acrylamino buffer solution pK 6.2	10 ml
01727 Acrylamino buffer pK 7.0	1g/5g
01729 Acrylamino buffer solution pK 7.0	10 ml
01735 Acrylamino buffer pK 8.5	1g/5g
01736 Acrylamino buffer solution 8.5	10 ml
01738 Acrylamino buffer solution pK 9.3	10 ml
01739 Acrylamino buffer pK 10.3	1g/5g
01741 Acrylamino buffer solution pK10.3	10 ml
01743 Acrylamino buffer solution pk >12.0	10 ml
84885 PI-Select [Acrylamino buffer set]	1 set

Instructions for use

A detailed protocol can be found in: *Immobilized pH Gradients: Theory and Methodology; Lab.Techniques in Biochemistry an Molecular Biology, vol. 20, P.G. Righetti, Elsevier Science Publishers, Amsterdam, The Netherlands, 1990, p 117 ff*

General Example of an IPG protocol

Table for preparing starting solutions for one or two gels:

Acrylamido buffer(s), 0.2M (*Volume in µl*)	Acrylamido buffer(s), 0.2M (*Volume in µl*)
Add water to a total vol. of 7.5 ml	Add water to a total vol. of 7.5 ml
Measure the pH with a pH meter.	Measure the pH with a pH meter.

Acrylamide/Bis (A) 2.0 ml

Glycerol (87%) 3.5 ml

Add water to a total volume of 15 ml

Acrylamide/Bis (A) 2.0 ml

Add water to a total volume of 15 ml

(A): From a stock 30% *T*, 4%

Note: TEMED and Ammonium persulfate are added after the transfer of the solutions to the mixing chambers: 15 µl Persulfate (40%); approx. 10 µl TEMED for 15 ml solution

Linear Gradient examples: volumes of acrylamido buffers for 15 ml each of starting solutions (2 gels)

pH range	Volume (*µl*) 0.2 M acrylamido buffer pK Acidic dense solution										Volume (*µl*) 0.2 M acrylamido buffer pK Basic light solution									
	1.0	3.1	3.6	4.6	6.2	7.0	8.5	9.3	10.3	>12	1.0	3.1	3.6	4.6	6.2	7.0	8.5	9.3	10.3	>12
3-7	556	35	44	13	284	218	-	-	-	-	-	636	132	157	19	195	-	-	818	-
3-9	1748	-	81	36	214	-	194	1273	-	-	-	938	66	316	12	161	74	232	-	1116
3-10	515	67	52	41	186	44	249	-	-	-	-	1011	200	375	13	147	105	127	15	1546
3-11	1492	-	64	46	147	31	209	193	842	-	-	915	184	372	16	139	56	14	107	1543
4-9	375	-	121	114	238	7	239	-	-	-	-	-	-	394	-	209	125	122	-	265
4-11	1127	-	52	131	165	15	247	178	583	-	-	-	-	400	13	188	39	52	116	458
6-11	-	785	-	-	193	14	146	114	392	-	-	-	-	-	148	304	34	59	78	60
2.5-11	1629	-	16	43	224	19	208	260	651	-	-	2182	376	506	36	224	108	71	79	3154
3-8	909	149	124	94	625	92	208	-	-	-	-	465	465	407	8	351	383	-	-	1010

References

Righetti P.G., Immobilized pH gradients: theory and methodology, in Laboratory techniques in biochemistry and molecular biology, **20**, Elsevier Science Publisher, Amsterdam, The Netherlands, 1990

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.