

How to Reproduce Natrix Reagents

Natrix reagents and optimization conditions based on Natrix hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of Natrix reagent 25 in a crystallization plate.

Solution Composition: 0.08 M Magnesium acetate tetrahydrate
0.05 M Sodium cacodylate trihydrate pH 6.5
30% w/v Polyethylene glycol 4,000

- 270 µl water³
- 80 µl 1.0 M Magnesium acetate tetrahydrate (CAS # 16674-78-5, Catalog # HR2-561)
- 50 µl 1.0 M Sodium cacodylate trihydrate pH 6.5 (CAS # 6131-99-3, Catalog # HR2-737)
- 600 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 1.0 milliliter of Natrix reagent 11.

Solution Composition: 0.01 M Magnesium chloride hexahydrate
0.05 M Sodium cacodylate trihydrate pH 6.0
1.0 M Lithium sulfate monohydrate

- 445 µl water³
- 50 µl 1.0 M Sodium cacodylate trihydrate pH 6.0 (CAS # 6131-99-3, Catalog # HR2-939-10)
- 5 µl 2.0 M Magnesium chloride hexahydrate (CAS # 7791-18-6, Catalog # HR2-559)
- 500 µl 2.0 M Lithium sulfate monohydrate (CAS # 10377-48-7, Catalog # HR2-545)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of Natrix reagent 48.

Solution Composition: 0.2 M Ammonium chloride
0.01 M Calcium chloride dihydrate
0.05 M TRIS hydrochloride pH 8.5
30% w/v Polyethylene glycol 4,000

- 3,050 µl water³

- 50 µl 2.0 M Calcium chloride dihydrate (CAS # 10035-04-8, Catalog # HR2-557)
- 400 µl 5.0 M Ammonium chloride (CAS # 12125-02-9, Catalog # HR2-691)
- 500 µl 1.0 M TRIS hydrochloride pH 8.5 (CAS # 1185-53-1, Catalog # HR2-727)
- 6,000 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well.

³ ASTM Type I water.

Formulation Notes for Natrix Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an Natrix reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from an Natrix kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

Table 1. Recommended reagents for the formulation of Natrix and Optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to

www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8
Ammonium chloride	HR2-691	5.0 M	200 ml	12125-02-9
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Lithium chloride	HR2-631	10.0 M	200 ml	7447-41-8
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium acetate tetrahydrate	HR2-561	1.0 M	100 ml	16674-78-5
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium sulfate heptahydrate	HR2-821	2.0 M	200 ml	10034-99-8
Magnesium sulfate hydrate	HR2-633	2.5 M	200 ml	22189-08-8
Potassium chloride	HR2-649	4.0 M	200 ml	7447-40-7
Sodium chloride	HR2-637	5.0 M	200 ml	7647-14-5
Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol 200	HR2-601	100	200 ml	25322-68-3
Polyethylene glycol 400	HR2-603	100%	200 ml	25322-68-3
Polyethylene glycol 4,000	HR2-529	50% w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50% w/v	200 ml	25322-68-3

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Table 1 (Continued). Recommended reagents for the formulation of Natrix and Optimization reagents.

Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol monomethyl ether 550	HR2-611	100%	200 ml	9004-74-4
Organics (non-volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
1,6-Hexanediol	HR2-625	6.0 M	200 ml	629-11-8
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100%	200 ml	107-41-5
Organics (non-volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
2-Propanol	HR2-619	100%	200 ml	67-63-0
Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
MES monohydrate pH 5.6 ²	HR2-943-05	1.0 M	185 ml	145224-94-8
MES monohydrate pH 6.0 ²	HR2-943-09	1.0 M	185 ml	145224-94-8
HEPES sodium pH 7.0 ¹	HR2-931-03	1.0 M	185 ml	75277-39-3
Sodium cacodylate trihydrate pH 6.0 ¹	HR2-939-10	1.0 M	185 ml	6131-99-3
Sodium cacodylate trihydrate pH 6.5 ¹	HR2-737	1.0 M	100 ml	6131-99-3
TRIS hydrochloride pH 7.5 ²	HR2-937-06	1.0 M	185 ml	1185-53-1
TRIS hydrochloride pH 8.5 ²	HR2-727	1.0 M	100 ml	1185-53-1
¹ pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0				
² pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2				

Table 2. Recommended buffers for screening the pH of Natrix and Optimization reagents.

Buffer Solution <u>or</u> Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
MES monohydrate <u>untitrated</u>	HR2-587	0.5 M	100 ml	145224-94-8	5.2 - 7.1
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ MES monohydrate kit ⁴	HR2-243	1.0 M	10 ml each	145224-94-8	5.2 - 7.1
HEPES sodium <u>untitrated</u>	HR2-577	1.0 M	100 ml	75277-39-3	6.6 - 8.5
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium HEPES kit ⁴	HR2-231	1.0 M	10 ml each	75277-39-3	6.8 - 8.2

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Table 2. Recommended buffers for screening the pH of Natrix and Optimization reagents.

Buffer Solution <u>or</u> Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Sodium cacodylate trihydrate <u>untitrated</u>	HR2-575	1.0 M	100 ml	6131-99-3	5.0 - 7.4
Titrated with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Cacodylate kit ⁴	HR2-239	1.0 M	10 ml each	6131-99-3	5.1 - 7.4
Tris hydrochloride <u>untitrated</u>	HR2-579	1.0 M	100 ml	1185-53-1	7.0 - 9.0
Titrated with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Tris Hydrochloride kit ⁴	HR2-237	1.0 M	10 ml each	1185-53-1	7.0 - 9.0
⁴ Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop					

Technical Support

Inquiries regarding Natrix Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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