GRAS Additive

HAMPTON RESEARCH
Solutions for Crystal Growth

Applications

GRAS Additive™ is an optimization kit designed to evaluate 96 unique water soluble reagents and their ability to influence, promote and improve the crystallization of biological macromolecules.

Features

- Developed at Hampton Research
- Bio focused additive screen for the optimization of biological macromolecular crystals
- For use with soluble proteins, membrane proteins, and biological therapeutics
- Generally Recognized As Safe reagent formulation
- Compatible with vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed GRAS Additive Reagent Formulation for more information.

General Description

GRAS Additive is an optimization kit designed to allow rapid and convenient evaluation of 96 unique reagents and their ability to influence the crystallization of biological macromolecules, including but not limited to soluble proteins, membrane proteins, and biological therapeutics.[1-5] The chemicals in GRAS Additive have been used under one or more of the following categories. As (1) a Generally Recognized As Safe (GRAS) substance, (2) a pharmaceutical excipient, (3) a normal physiological constituent, (4) a metabolic byproduct[5], and/or (5) a Everything Added to Food in the United States (EAFUS) substance.

The 96 by 1 ml, deep well block reagent screen, is designed to be compatible with most popular crystallization reagents including reagents utilized in Hampton Research screens. Compatible with vapor diffusion, microbatch, and free interface diffusion. For research use only.

Sample Preparation

The protein sample should be homogenous, as pure as is practically possible (>95%), and free of amorphous material. Remove amorphous material by centrifugation or microfiltration prior to use. The recommended sample concentration is 5 to 25 mg/ml in dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the GRAS Additive reagents. However, agents that promote and preserve sample solubility, stability, and homogeneity can and should be included in the sample buffer. For additional sample preparation recommendations see Hampton Research Crystal Growth 101 – Sample Preparation for Crystallization.

Preparing the Deep Well Block for Use

Allow the Deep Well Block and reagents to stabilize at room temperature. If reagents precipitate during cold storage, warm the sealed block at up to 50°C for up to 60 minutes, inverting the block several times to solubilize the reagents. Centrifuge the block at 500 rpm for 5 minutes to remove stray drops from the film before removing the sealing film. The film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and pierced to access reagents. For storage, reseal using AlumaSeal II Sealing Film.

Performing the Screen

Automated Method - Sitting Drop Vapor Diffusion

The GRAS Additive deep well block is compatible with the SBS standard 96 well microplate format and is compatible with numerous automated liquid handling systems that accept 8 x 12, 96 well assay blocks. Follow the automation manufacturer's recommendation for handling Deep Well blocks.

1. Using a 96 well sitting drop vapor diffusion plate, dispense the recommended volume (typically 45 to 90 microliters) of crystallization reagent used to produce the desired crystals into the reagent reservoirs of the crystallization plate.

2. Dispense a volume of GRAS Additive into each reagent well to produce a 1:10 dilution of the GRAS Additive reagent and mix thoroughly. For a 50 µl reservoir volume, pipette 5 µl of GRAS Additive plus 45 µl of crystallization reagent into the reservoir. For a 100 µl reservoir volume, pipette 10 µl of GRAS Additive plus 90 µl of crystallization reagent into the reservoir.

3. Dispense the desired volume of crystallization reagent (typically 50 to 200 nanoliters) from the crystallization plate reservoir to the sitting drop well.

4. Transfer the equivalent volume of sample to the reagent drop in the sitting drop well.

5. Seal the crystallization plate using a clear sealing tape or film. View and score the experiment. See Hampton Research Crystal Growth 101 - Viewing Crystallization Experiments for more information.

6. Seal the remaining GRAS Additive reagent in the Deep Well block using AlumaSeal II Sealing Film.

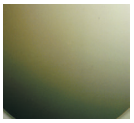
Manual Method - Sitting Drop Vapor Diffusion

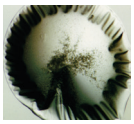
The GRAS Additive deep well block is compatible with the SBS standard 96 well microplate format and is compatible with numerous 8, 12, and 96 channel automated and manual pipettes.

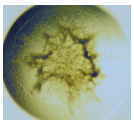
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 45 to 90 microliters) of crystallization reagent used to produce the desired crystals into the reagent reservoirs of the crystallization plate.

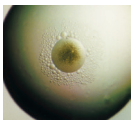
2. Use clean pipet tips for each reagent set, transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer GRAS Additive reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns 2 through 12. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer GRAS Additive reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows B through H. Dispense a volume of GRAS Additive into each reagent well to produce a 1:10 dilution of the GRAS Additive reagent and mix thoroughly. For a 50 µl reservoir volume, pipette 5 µl of GRAS Additive plus 45 µl of crystallization reagent into the reservoir. For a 100 µl reservoir volume, pipette 10 µl of GRAS Additive plus 90 µl of crystallization reagent into the reservoir.

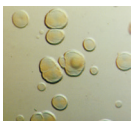
Figure 1
Typical observations in a crystallization experiment

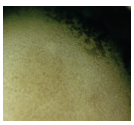

Clear Drop


Skin / Precipitate

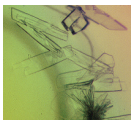

Precipitate


Precipitate / Phase

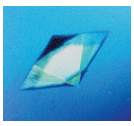

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

3. Using clean pipet tips, pipet the desired volume of crystallization reagent (typically 0.05 to 2 microliters) from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents.

4. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.

5. Seal the crystallization plate using an optically clear sealing film or tape. Seal the remaining GRAS Additive reagent in the Deep Well block using AlumaSeal II sealing film.

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after for up to 60 days, or until the drop dries out. Records should indicate whether the drop is clear, contains precipitate, and/or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a numerical scoring scheme (Clear = 0, Crystal = 1. Precipitate = 2). Figure 1 shows typical examples of what one might observe in a crystallization experiment. For more information see Crystal Growth 101 – Viewing Crystallization Experiments.

Interpreting GRAS Additive

Additives are considered chemicals in a crystallization reagent in addition to the primary precipitant, secondary reagent, and buffer. Additives can affect the solubility and crystallizability of biological macromolecules. These chemicals can perturb, manipulate, and stabilize sample-sample and sample-solvent interactions, as well as perturb water structure, which can alter and improve the solubility and crystallization of the protein. Additives can stabilize or engender conformity by specific interactions with the protein. Additives can also establish stabilizing, intermolecular, non-covalent crosslinks in protein crystals and thereby promote lattice formation.

The most commonly useful class of additives, and the only class of which we have any real understanding, are those which may, for physiological reasons, be bound by the protein with consequent favorable change in the protein physical-chemical properties or conformation. These include coenzymes and prosthetic groups, inhibitors, enzymatic products, ions, and other effector molecules. Often times the ligand bound form of the protein is structurally defined and stable, while the unliganded form is not, and often the former will crystallize when the latter will not.

Review and compare the result of each GRAS Additive reagent with the crystals originally produced with only the original crystallization reagent, free of additive. Results can be explored further by screening the concentration of the additive, drop ratio, temperature, and other crystallization variables. For more information about crystal optimization, see Crystal Growth 101 – Optimization.

GRAS Additive Formulation & Storage

Each of the reagents is formulated in Type 1+ ultrapure water (18.2 megaohm-cm resistivity at 25°C, < 5 ppb Total Organic Carbon, bacteria free (<1 Bacteria (CFU/ml)), pyrogen free (<0.03 Endotoxin (EU/ml)), RNase-free (< 0.01 ng/mL) and DNase-free (< 4 pg/µL)) and sterile filtered using a 0.2 micron filter. Recommended storage for GRAS Additive is -20°C. Best if used within 12 months of receipt.

Recommended Reading

1. Searching for silver bullets: An alternative strategy for crystallizing macromolecules. Alexander McPherson and Bob Cudney. Journal of Structural Biology 156 (2006) 387-406.
2. A novel strategy for the crystallization of proteins: X-ray diffraction validation. Steven B. Larson, John S. Day, Robert Cudney, and Alexander McPherson. Acta Cryst. (2007) D63, 310-318.
3. Development of an alternative approach to protein crystallization. McPherson, Alexander; Nguyen, Chieniang; Larson, Steven B; Day, John S; Cudney, Bob. J Struct Funct Genomics, Volume 8, Number 4, December 2007, 193-198.
4. Progress in the Development of an Alternative Approach to Macromolecular Crystallization. S. B. Larson,J. S. Day, C. Nguyen, R. Cudney, and A. McPherson. Crystal Growth & Design 2008 Volume 8, No. 8 3038-3052.
5. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, et al., HMDB 3.0 — The Human Metabolome Database in 2013. Nucleic Acids Res. 2013. Jan 1;41(D1):D801-7. 23161693
6. Optimization of crystallization conditions for biological macromolecules. Alexander McPherson and Bob Cudney. Acta Crystallographica Section F Volume 70, Issue 11, pages 1445–1467, November 2014.
7. Screening and optimization strategies for macromolecular crystal growth. Cudney R, Patel S, Weisgraber K, Newhouse Y, McPherson A. Acta Crystallogr D Biol Crystallogr. 1994 Jul 1;50(Pt 4):414-23.

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Well #	Additive	Classification
1. (A1)	30 mM Nα-Benzoyl-L-arginine ethyl ester hydrochloride	Amine
2. (A2)	40 mM L-Carnosine	Amine
3. (A3)	70 mM Choline chloride	Amine
4. (A4)	50 mM Histamine dihydrochloride	Amine
5. (A5)	110 mM Sarcosine	Amine
6. (A6)	0.05% w/v L-Aspartic acid, 0.05% w/v L-Glutamic acid	Amino acid
7. (A7)	0.5% w/v Glycine, 0.5% w/v L-Proline	Amino acid
8. (A8)	0.33% w/v L-Arginine, 0.33% w/v L-Histidine, 0.33% w/v L-Lysine	Amino acid
9. (A9)	0.25% w/v L-Asparagine monohydrate, 0.25% w/v L-Glutamine, 0.25% w/v L-Serine, 0.25% w/v L-Threonine	Amino acid
10. (A10)	0.0125% w/v L-Alanine, 0.0125% w/v L-Isoleucine, 0.0125% w/v L-Leucine, 0.0125% w/v L-Methionine, 0.0125% w/v L-Phenylalanine, 0.0125% w/v L-Tyrosine, 0.0125% w/v L-Tryptophan, 0.0125% w/v L-Valine	Amino acid
11. (A11)	0.05% w/v L-Alanine, 0.05% w/v L-Arginine, 0.05% w/v L-Asparagine monohydrate, 0.05% w/v L-Aspartic acid, 0.05% w/v Glycine, 0.05% w/v L-Glutamic acid, 0.05% w/v L-Glutamine, 0.05% w/v L-Histidine, 0.05% w/v L-Isoleucine, 0.05% w/v L-Leucine, 0.05% w/v L-(+)-Lysine, 0.05% w/v L-Methionine, 0.05% w/v L-Phenylalanine, 0.05% w/v L-Proline, 0.05% w/v L-Serine, 0.05% w/v L-(-)-Threonine, 0.05% w/v L-Tryptophan, 0.05% w/v L-Tyrosine, 0.05% w/v L-Valine	Amino acid
12. (A12)	50 mM N-Acetyl-L-glutamic acid	Amino acid derivative
13. (B1)	60 mM L-Citrulline	Amino acid derivative
14. (B2)	80 mM L-Homoserine	Amino acid derivative
15. (B3)	80 mM 4-Hydroxy-L-proline	Amino acid derivative
16. (B4)	60 mM L-Ornithine hydrochloride	Amino acid derivative
17. (B5)	80 mM L-Pyroglutamic acid	Amino acid derivative
18. (B6)	50 mM N-Acetyl-D-glucosamine	Amino sugar
19. (B7)	50 mM N-Acetyl-D-mannosamine	Amino sugar
20. (B8)	5 mM D-(+)-Galactosamine hydrochloride	Amino sugar
21. (B9)	50 mM D-(+)-Glucosamine hydrochloride	Amino sugar
22. (B10)	5% w/v n-Dodecyl-β-D-maltoside	Detergent
23. (B11)	5% w/v Lauryldimethylamine-N-oxide	Detergent
24. (B12)	5% w/v n-Octyl-β-D-glucoside	Detergent
25. (C1)	10% w/v TWEEN® 20	Detergent
26. (C2)	10% w/v TWEEN® 80	Detergent
27. (C3)	70 mM Calcium chloride dihydrate	Metal
28. (C4)	50 mM Magnesium chloride hexahydrate	Metal
29. (C5)	70 mM Zinc chloride	Metal
30. (C6)	60 mM cis-Aconitic acid	Organic acid
31. (C7)	7 mM 4-Aminobenzoic acid	Organic acid
32. (C8)	70 mM Creatine monohydrate	Organic acid
33. (C9)	9 mM Fumaric acid	Organic acid
34. (C10)	40 mM DL-Isocitric acid trisodium salt	Organic acid
35. (C11)	40 mM α-Ketoglutaric acid disodium salt	Organic acid
36. (C12)	110 mM L-(+)-Lactic acid	Organic acid
37. (D1)	80 mM DL-Malic acid	Organic acid
38. (D2)	80 mM Oxaloacetic acid	Organic acid
39. (D3)	110 mM Pyruvic acid	Organic acid
40. (D4)	50 mM Sodium gluconate	Organic acid
41. (D5)	90 mM Succinic acid	Organic acid
42. (D6)	7 mM Vanillin	Organic acid
43. (D7)	30 mM Mellitic acid	Organic acid
44. (D8)	50 mM Gly-gly-gly	Peptide
45. (D9)	30 mM Creatine phosphate disodium salt tetrahydrate	Phosphate
46. (D10)	30 mM α-D-Glucose 1-phosphate disodium salt hydrate	Phosphate
47. (D11)	40 mM Sodium phenyl phosphate dibasic dihydrate	Phosphate
48. (D12)	20% v/v Glycerol	Polyol

Reagents formulated in Type 1+ ultrapure grade water, no pH adjustment.

Well #	Additive	Classification
49. (E1)	20% v/v Polyethylene glycol 200	Polyol
50. (E2)	1% v/v Propylene glycol	Polyol
51. (E3)	1% w/v (+)-Arabinogalactan	Polysaccharide
52. (E4)	1% w/v Chondroitin sulfate A sodium salt	Polysaccharide
53. (E5)	1% w/v Dextran 1,500	Polysaccharide
54. (E6)	1% w/v Dextran 6,000	Polysaccharide
55. (E7)	1% w/v Dextran sulfate sodium salt 4,000	Polysaccharide
56. (E8)	1% w/v Dextran sulfate sodium salt 8,000	Polysaccharide
57. (E9)	1% w/v Dextran sulfate sodium salt 15,000	Polysaccharide
58. (E10)	1% w/v Fructooligosaccharides	Polysaccharide
59. (E11)	4 mM Adenosine	Purine
60. (E12)	50 mM Caffeine	Purine
61. (F1)	0.4 mM Guanosine	Purine
62. (F2)	40 mM Inosine	Purine
63. (F3)	40 mM Cytidine	Pyrimidine
64. (F4)	9 mM Cytosine	Pyrimidine
65. (F5)	40 mM Thymidine	Pyrimidine
66. (F6)	8 mM Thymine	Pyrimidine
67. (F7)	40 mM Uridine	Pyrimidine
68. (F8)	1,000 mM Sodium chloride	Salt
69. (F9)	3,000 mM NDSB-195	Solubilizing Agent
70. (F10)	250 mM L-(+)-Arabinose	Sugar
71. (F11)	250 mM L-(–)-Arabitol	Sugar
72. (F12)	250 mM D-(+)-Fucose	Sugar
73. (G1)	110 mM DL-Glyceraldehyde	Sugar
74. (G2)	30 mM D-Lactose monohydrate	Sugar
75. (G3)	60 mM D-Mannitol	Sugar
76. (G4)	60 mM D-(+)-Mannose	Sugar
77. (G5)	250 mM D-(-)-Ribose	Sugar
78. (G6)	250 mM D-(+)-Sucrose	Sugar
79. (G7)	250 mM D-(+)-Trehalose dihydrate	Sugar
80. (G8)	250 mM DL-Xylose	Sugar
81. (G9)	50 mM Disodium β-Glycerophosphate tetrahydrate	Sugar phosphate
82. (G10)	30 mM D-Fructose 1,6-diphosphate trisodium salt hydrate	Sugar phosphate
83. (G11)	40 mM Isopropyl 1-thio-β-D-galactopyranoside	Sugar derivative
84. (G12)	60 mM L-Ascorbic acid	Vitamin
85. (H1)	0.4 mM Biotin	Vitamin
86. (H2)	80 mM Niacin	Vitamin
87. (H3)	80 mM Nicotinamide	Vitamin
88. (H4)	50 mM DL-Panthenol	Vitamin
89. (H5)	40 mM D-Pantothenic acid hemicalcium salt	Vitamin
90. (H6)	50 mM Pyridoxal hydrochloride	Vitamin
91. (H7)	40 mM Pyridoxamine dihydrochloride	Vitamin
92. (H8)	60 mM Pyridoxine	Vitamin
93. (H9)	4 mM Pyridoxal 5-phosphate monohydrate	Vitamin
94. (H10)	30 mM Thiamine hydrochloride	Vitamin
95. (H11)	20 mM Thiamine pyrophosphate	Vitamin
96. (H12)	20 mM Thiamine monophosphate chloride dihydrate	Vitamin derivative

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Reagents formulated in Type 1+ ultrapure grade water, no pH adjustment.

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

GRAS Additive™ - HR2-459 Scoring Sheet		Date:	Date:	Date:
1. (A1)	30 mM Nα-Benzoyl-L-arginine ethyl ester hydrochloride			
2. (A2)	40 mM L-Carnosine			
3. (A3)	70 mM Choline chloride			
4. (A4)	50 mM Histamine dihydrochloride			
5. (A5)	110 mM Sarcosine			
6. (A6)	0.05% w/v L-Aspartic acid, 0.05% w/v L-Glutamic acid			
7. (A7)	0.5% w/v Glycine, 0.5% w/v L-Proline			
8. (A8)	0.33% w/v L-Arginine, 0.33% w/v L-Histidine, 0.33% w/v L-Lysine			
9. (A9)	0.25% w/v L-Asparagine monohydrate, 0.25% w/v L-Glutamine, 0.25% w/v L-Serine, 0.25% w/v L-Threonine			
10. (A10)	0.0125% w/v L-Alanine, 0.0125% w/v L-Isoleucine, 0.0125% w/v L-Leucine, 0.0125% w/v L-Methionine, 0.0125% w/v L-Phenylalanine, 0.0125% w/v L-Tyrosine, 0.0125% w/v L-Tryptophan, 0.0125% w/v L-Valine			
11. (A11)	0.05% w/v L-Alanine, 0.05% w/v L-Arginine, 0.05% w/v L-Asparagine monohydrate, 0.05% w/v L-Aspartic acid, 0.05% w/v Glycine, 0.05% w/v L-Glutamic acid, 0.05% w/v L-Glutamine, 0.05% w/v L-Histidine, 0.05% w/v L-Isoleucine, 0.05% w/v L-Leucine, 0.05% w/v L-(+)-Lysine, 0.05% w/v L-Methionine, 0.05% w/v L-Phenylalanine, 0.05% w/v L-Proline, 0.05% w/v L-Serine, 0.05% w/v L-(-)-Threonine, 0.05% w/v L-Tryptophan, 0.05% w/v L-Tyrosine, 0.05% w/v L-Valine			
12. (A12)	50 mM N-Acetyl-L-glutamic acid			
13. (B1)	60 mM L-Citrulline			
14. (B2)	80 mM L-Homoserine			
15. (B3)	80 mM 4-Hydroxy-L-proline			
16. (B4)	60 mM L-Ornithine hydrochloride			
17. (B5)	80 mM L-Pyroglutamic acid			
18. (B6)	50 mM N-Acetyl-D-glucosamine			
19. (B7)	50 mM N-Acetyl-D-mannosamine			
20. (B8)	5 mM D-(+)-Galactosamine hydrochloride			
21. (B9)	50 mM D-(+)-Glucosamine hydrochloride			
22. (B10)	5% w/v n-Dodecyl-β-D-maltoside			
23. (B11)	5% w/v Lauryldimethylamine-N-oxide			
24. (B12)	5% w/v n-Octyl-β-D-glucoside			
25. (C1)	10% w/v TWEEN® 20			
26. (C2)	10% w/v TWEEN® 80			
27. (C3)	70 mM Calcium chloride dihydrate			
28. (C4)	50 mM Magnesium chloride hexahydrate			
29. (C5)	70 mM Zinc chloride			
30. (C6)	60 mM cis-Aconitic acid			
31. (C7)	7 mM 4-Aminobenzoic acid			
32. (C8)	70 mM Creatine monohydrate			
33. (C9)	9 mM Fumaric acid			
34. (C10)	40 mM DL-Isocitric acid trisodium salt			
35. (C11)	40 mM α-Ketoglutaric acid disodium salt			
36. (C12)	110 mM L-(+)-Lactic acid			
37. (D1)	80 mM DL-Malic acid			
38. (D2)	80 mM Oxaloacetic acid			
39. (D3)	110 mM Pyruvic acid			
40. (D4)	50 mM Sodium gluconate			
41. (D5)	90 mM Succinic acid			
42. (D6)	7 mM Vanillin			
43. (D7)	30 mM Mellitic acid			
44. (D8)	50 mM Gly-gly-gly			
45. (D9)	30 mM Creatine phosphate disodium salt tetrahydrate			
46. (D10)	30 mM α-D-Glucose 1-phosphate disodium salt hydrate			
47. (D11)	40 mM Sodium phenyl phosphate dibasic dihydrate			
48. (D12)	20% v/v Glycerol			

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Solutions for Crystal Growth

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Sample: _____ **Sample Concentration:** _____

Sample Buffer: _____ **Date:** _____

Reservoir Volume: _____ **Temperature:** _____

Drop Volume: Total _____ µl **Sample** _____ µl **Reservoir** _____ µl **Additive** _____ µl

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

GRAS Additive™ - HR2-459 Scoring Sheet	Date:	Date:	Date:
49. (E1) 20% v/v Polyethylene glycol 200			
50. (E2) 1% v/v Propylene glycol			
51. (E3) 1% w/v (+)-Arabinogalactan			
52. (E4) 1% w/v Chondroitin sulfate A sodium salt			
53. (E5) 1% w/v Dextran 1,500			
54. (E6) 1% w/v Dextran 6,000			
55. (E7) 1% w/v Dextran sulfate sodium salt 4,000			
56. (E8) 1% w/v Dextran sulfate sodium salt 8,000			
57. (E9) 1% w/v Dextran sulfate sodium salt 15,000			
58. (E10) 1% w/v Fructooligosaccharides			
59. (E11) 4 mM Adenosine			
60. (E12) 50 mM Caffeine			
61. (F1) 0.4 mM Guanosine			
62. (F2) 40 mM Inosine			
63. (F3) 40 mM Cytidine			
64. (F4) 9 mM Cytosine			
65. (F5) 40 mM Thymidine			
66. (F6) 8 mM Thymine			
67. (F7) 40 mM Uridine			
68. (F8) 1,000 mM Sodium chloride			
69. (F9) 3,000 mM NDSB-195			
70. (F10) 250 mM L-(+)-Arabinose			
71. (F11) 250 mM L-(–)-Arabitol			
72. (F12) 250 mM D-(+)-Fucose			
73. (G1) 110 mM DL-Glyceraldehyde			
74. (G2) 30 mM D-Lactose monohydrate			
75. (G3) 60 mM D-Mannitol			
76. (G4) 60 mM D-(+)-Mannose			
77. (G5) 250 mM D-(-)-Ribose			
78. (G6) 250 mM D-(+)-Sucrose			
79. (G7) 250 mM D-(+)-Trehalose dihydrate			
80. (G8) 250 mM DL-Xylose			
81. (G9) 50 mM Disodium β-Glycerophosphate tetrahydrate			
82. (G10) 30 mM D-Fructose 1,6-diphosphate trisodium salt hydrate			
83. (G11) 40 mM Isopropyl 1-thio-β-D-galactopyranoside			
84. (G12) 60 mM L-Ascorbic acid			
85. (H1) 0.4 mM Biotin			
86. (H2) 80 mM Niacin			
87. (H3) 80 mM Nicotinamide			
88. (H4) 50 mM DL-Panthenol			
89. (H5) 40 mM D-Pantothenic acid hemicalcium salt			
90. (H6) 50 mM Pyridoxal hydrochloride			
91. (H7) 40 mM Pyridoxamine dihydrochloride			
92. (H8) 60 mM Pyridoxine			
93. (H9) 4 mM Pyridoxal 5-phosphate monohydrate			
94. (H10) 30 mM Thiamine hydrochloride			
95. (H11) 20 mM Thiamine pyrophosphate			
96. (H12) 20 mM Thiamine monophosphate chloride dihydrate			

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