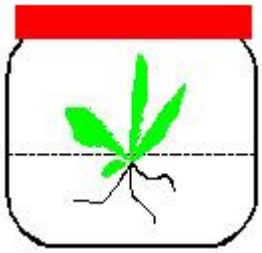

Kitchen Culture Kits Inc.

Protocols for the Hobbyist

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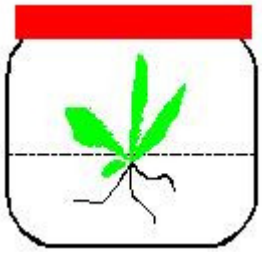

"Bringing plant tissue culture to the classroom and home since 1998"

NOTE: All protocols are intended to be used following the methods described in the KCK Manual: "Plant Tissue Culture for the Classroom and Home" or the online workshop handout.

Plant Hormone/Growth Regulator Summary

HORMONE	MW	Milligrams in 1 µM	Milligrams in 2 µM	Milligrams in 4 µM	1 mg in µM
BAP	225	.225	.45	.90	4.44 µM
KINETIN	215	.215	.43	.86	4.65 µM
2iP	203	.203	.41	.82	4.82 µM
TDZ	220	.220	.44	.88	4.55 µM
Zeatin	219	.219	.44	.88	4.55 µM
IAA	175	.175	.35	.70	5.71 µM
IBA	203	.203	.40	.80	4.92 µM
NAA	186	.186	.37	.74	5.37 µM
24D	221	.221	.44	.88	4.55 µM
GA3	346	.366	.73	1.46	2.89 µM

Plant hormones/growth regulators vary in their stability.

- Powdered forms of BAP and NAA are stored at room temperature.
- All other powders should be stored frozen.
- It is recommended that all powdered forms be shipped via priority mail.
- Solutions vary in their stability.
  - BAP, kinetin and NAA solutions appear to be stable at room temperature for less than 30 days but should be refrigerated as soon as possible.
  - IAA is not stable in light and will degrade within 3 days. Make fresh and keep refrigerated.
  - Other solutions need to be refrigerated or frozen. Shipping of these solutions should be avoided unless shipped within 3 days. These are best purchased in powder form and made in solution on site followed by refrigeration.

BAP Dilutions

The molecular weight of BAP, benzylaminopurine, is 225

Molarity per liter		
1 M	=	225 grams
0.1 M	=	22.5 grams
0.01 M	=	2.25 grams
0.001 M (1 mM)	=	0.225 grams (225 mg)
0.0001 M (0.1 mM)	=	0.0225 grams (22.5 mg)
0.00001 M (0.01 mM)	=	0.00225 grams (2.25 mg)
0.000001 M (1 µM)	=	0.000225 grams (0.225 mg)

NOTE: Therefore, a solution that is 2.22 µM BAP is 2.22 * 0.225 mg BAP = 0.4495 mg BAP, and this is rounded to 0.5 mg BAP.

If the BAP stock solution = 1 mg/ml, then using 0.5 ml = 0.5 mg

Desired concentration of BAP	Volume of BAP stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.225 mg	1 ml contains 1 mg BAP so 0.225 ml contains 0.225 mg BAP
2 µM = 0.45 mg	= 0.450 ml
3 µM = 0.675 mg	= 0.675 ml
4 µM = 0.90 mg	= 0.900 ml
4.44 uM = 0.99 mg	= 1.000 ml

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KINETIN Dilutions

The molecular weight of kinetin, 6-Furfurylaminopurine, is 215.21

Molarity per liter		
1 M	=	215.21 grams
0.1 M	=	21.52 grams
0.01 M	=	2.152 grams
0.001 M (1 mM)	=	0.2152 grams (215 mg)
0.0001 M (0.1 mM)	=	0.0215 grams (21.5 mg)
0.00001 M (0.01 mM)	=	0.00215 grams (2.15 mg)
0.000001 M (1 µM)	=	0.000215 grams (0.215 mg)

NOTE: Therefore, a solution that is 2.22 µM kinetin is calculated:
2.22 * 0.215 mg kinetin = 0.4773 mg kinetin, and
this is rounded to 0.5 mg kinetin.

If the kinetin stock solution = 1 mg/ml, then using 0.5 ml = 0.5 mg

Desired concentration of kinetin	Volume of kinetin stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.215 mg	1 ml contains 1 mg kinetin Therefore 0.215 ml contains 0.215 mg kinetin
2 µM = 0.430 mg	= 0.43 ml
3 µM = 0. 645 mg	= 0.65 ml
4 µM = 0.860 mg	= 0.86 ml
4.65 µM = 0.9998 mg	= 1.000 ml

TDZ Dilutions

The molecular weight of TDZ, Thidiazuron [1-phenyl-3-(1, 2, 3-thidiazol-5-yl) urea], is 220.25

Molarity per liter		
1 M	=	220.25 grams
0.1 M	=	22.03 grams
0.01 M	=	2.20 grams
0.001 M (1 mM)	=	0.220 grams (220 mg)
0.0001 M (0.1 mM)	=	0.0220 grams (22.0 mg)
0.00001 M (0.01 mM)	=	0.00220 grams (2.20 mg)
0.000001 M (1 µM)	=	0.000220 grams (0.220 mg)

NOTE: Therefore, a solution that is 2.22 µM TDZ is 2.22 * 0.220 mg TDZ = 0.4884 mg, and this is rounded to 0.5 mg TDZ.

If the TDZ stock solution = 1 mg/ml, then using 0.5 ml = 0.5 mg

Desired concentration of TDZ	Volume of TDZ stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.220 mg	1 ml contains 1 mg TDZ Therefore 0.220 ml contains 0.220 mg TDZ
2 µM = 0.44 mg	= 0.44 ml
3 µM = 0.66 mg	= 0.66 ml
4 µM = 0.88 mg	= 0.88 ml
4.55 µM = 1.00 mg	= 1.000 ml

Zeatin Dilutions

The molecular weight of zeatin is 219.25

Molarity per liter

1 M	=	219.25 grams
0.1 M	=	21.93 grams
0.01 M	=	2.19 grams
0.001 M (1 mM)	=	0.219 grams (219 mg)
0.0001 M (0.1 mM)	=	0.0219 grams (21.9 mg)
0.00001 M (0.01 mM)	=	0.0022 grams (2.19 mg)
0.000001 M (1 µM)	=	0.000219 grams (0.219 mg)

NOTE: Therefore, a solution that is 2.22 µM zeatin is 2.22 * 0.219 mg = 0.4862 mg, and this is rounded to 0.5 mg zeatin.

If the zeatin stock solution = 1 mg/ml, then using 0.4 ml = 0.4 mg

Desired concentration of zeatin	Volume of zeatin stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.219 mg	1 ml contains 1 mg zeatin Therefore 0.219 ml contains 0.219 mg zeatin
2 µM = 0.43 mg	= 0.43 ml
3 µM = 0.65 mg	= 0.65 ml
4 µM = 0.86 mg	= 0.86 ml
4.55 µM = 0.999 mg	= 1.000 ml

2iP

The molecular weight of 2iP, 6-(y,y-DIMETHYLALLYLAMINO)PURINE, is 203.3

Molarity per liter

1 M	=	203.3 grams
0.1 M	=	20.33 grams
0.01 M	=	2.03 grams
0.001 M (1 mM)	=	0.203 grams (203 mg)
0.0001 M (0.1 mM)	=	0.0203 grams (20.3 mg)
0.00001 M (0.01 mM	=	0.00203 grams (2.03 mg)
0.000001 M (1 µM)	=	0.000203 grams (0.203 mg)

NOTE: Therefore, a solution that is 2.22 µM 2iP is 2.22 * 0.203 mg 2iP = 0.4507 mg, and this is rounded to 0.5 mg 2iP

If the 2iP stock solution = 1 mg/ml, then using 0.5 ml = 0.5 mg

Desired concentration of 2iP	Volume of 2iP stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.203 mg	1 ml contains 1 mg 2iP Therefore 0.203 ml contains 0.203 mg 2iP
2 µM = 0.41 mg	= 0.41 ml
3 µM = 0.61 mg	= 0.61 ml
4 µM = 082 mg	= 0.82 ml
4.821 µM = 0.999 mg	= 1.000 ml

IAA Dilutions

The molecular weight of IAA, indole-3-acetic acid, is 175.19

Molarity per liter

1 M = 175.19 grams
0.1 M = 17.52 grams
0.01 M = 1.75 grams
. 001 M (1 mM) = 0.175 grams (175 mg)
0001 M (0.1 mM) = 0.0175 grams (17.5 mg)
0.00001 M (0.01 mM) = 0.00175 grams (1.75 mg)
0.000001 M (1 µM) = 0.000175 grams (0.175 mg)

NOTE: Therefore, a solution that is 2.22 µM IAA is 2.22 * 0.175 mg IAA = 0.3885 mg, and this is rounded to 0.4 mg IAA

If the IAA stock solution = 1 mg/ml, then using 0.4 ml = 0.4 mg

Desired concentration of IAA	Volume of IAA stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.175 mg	1 ml contains 1 mg IAA Therefore 0.175 ml contains 0.175 mg IAA
2 µM = 0.35 mg	= 0.35 ml
3 µM = 0.525 mg	= 0.53 ml
4 µM = 0.70 mg	= 0.70 ml
5.71 µM = 0.999 mg	= 1.000 ml

IBA Dilutions

The molecular weight of IBA, indole-3-butyric acid is 203.24

Molarity per liter

1 M	=	203.24 grams
0.1 M	=	20.34 grams
0.01 M	=	2.03 grams
0.001 M (1 mM)	=	0.203 grams (203 mg)
0.0001 M (0.1 mM)	=	0.0203 grams (20.3 mg)
0.00001 M (0.01 mM)	=	0.00203 grams (2.03 mg)
0.000001 M (1 µM)	=	0.000203 grams (0.203 mg)

NOTE: Therefore, a solution that is 2.22 µM IBA is 2.22 * 0.203 mg IBA = 0.4507 mg, and this is rounded to 0.5 mg IBA.

If the IBA stock solution = 1 mg/ml, then using 0.4 ml = 0.4 mg

Desired concentration of IBA	Volume of IBA stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.203 mg	1 ml contains 1 mg IBA Therefore 0.203 ml contains 0.203 mg IBA
2 µM = 0.41 mg	= 0.41 ml
3 µM = 0.61 mg	= 0.61 ml
4 µM = 0.82 mg	= 0.82 ml
4.92 µM = 0.999 mg	= 1.000 ml

NAA Dilutions

The molecular weight of NAA, naphthalene acidic acid, is 186.2

Molarity per liter

1 M	=	186.2 grams
0.1 M	=	18.62 grams
0.01 M	=	1.86 grams
0.001 M (1 mM)	=	0.186 grams (186 mg)
0.0001 M (0.1 mM)	=	0.0186 grams (18.6 mg)
0.00001 M (0.01 mM)	=	0.00186 grams (1.86 mg)
0.000001 M (1 µM)	=	0.000186 grams (0.186 mg)

NOTE: Therefore, a solution that is 2.22 µM NAA is: 2.22 * 0.186 mg NAA = 0.4129 mg, and this is rounded to 0.4 mg NAA.

If the NAA stock solution = 1 mg/ml, then using 0.4 ml = 0.4 mg

Desired concentration of NAA	Volume of NAA stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.186 mg	1 ml contains 1 mg NAA Therefore 0.186 ml contains 0.186 mg NAA
2 µM = 0.370 mg	= 0.37 ml
3 µM = 0. 558 mg	= 0.56 ml
4 µM = 0.740 mg	= 0.74 ml
5.37 µM = 0.999 mg	= 1.000 ml

2,4-D Dilutions

The molecular weight of 24D, 2,4-dichlorophenoxyacetic acid, is 221.04

Molarity per liter		
1 M	=	221.04 grams
0.1 M	=	22.1 grams
0.01 M	=	2.21 grams
0.001 M (1 mM)	=	0.221 grams (221 mg)
0.0001 M (0.1 mM)	=	0.0221 grams (22.1 mg)
0.00001 M (0.01 mM)	=	0.00221 grams (2.21 mg)
0.000001 M (1 µM)	=	0.000221 grams (0.221 mg)

NOTE: Therefore, a solution that is 2.22 µM 24D is 2.22 * 0.221 mg TDZ = 0.4906 mg, and this is rounded to 0.5 mg 24D.

If the 24D stock solution = 1 mg/ml, then using 0.5 ml = 0.5 mg

Desired concentration of 24D	Volume of TDZ stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.221 mg	1 ml contains 1 mg 24D Therefore 0.220 ml contains 0.220 mg 24D
2 µM = 0.44 mg	= 0.44 ml
3 µM = 0.66 mg	= 0.66 ml
4 µM = 0.88 mg	= 0.88 ml
4.55 µM = 1.00 mg	= 1.000 ml

GA3 Dilutions

The molecular weight of GA3, gibberellic acid 3, is 346.4

1 M	=	346.4 gram
0.1 M	=	34.6 grams
0.01 M	=	3.46 grams
0.001 M (1 mM)	=	0.346 grams (346 mg)
0.0001 M (0.1 mM)	=	0.0346 grams (34.6 mg)
0.00001 M (0.01 mM)	=	0.00346 grams (3.46 mg)
0.000001 M (1 µM)	=	0.000346 grams (0.346 mg)

NOTE: Therefore, a solution that is 2.22 µM GA3 is: 2.22 * 0.386 mg GA3 = 0.8492 mg, and this is rounded to 0.9 mg GA3.

If the GA3 stock solution = 1 mg/ml, then using 0.9 ml = 0.9 mg

*****NOTE: GA3 stock solution is often made at 13 mg/ml*****

Desired concentration of GA3	Volume of GA3 stock solution (1 mg/ml) to add for 1 liter medium
1 µM = 0.346 mg	1 ml contains 1 mg GA3 Therefore 0.346 ml contains 0.346 mg GA3
2 µM = 0.692 mg	= 0.69 ml
3 µM = 1.038 mg	= 1.04 ml
4 µM = 1.384 mg	= 1.38 ml
2.89 µM = 0.999 mg	= 1.000 ml