Chemical Resources—Reagent Preparation

Almost all research projects are limited by inadequate resources. Limitations may result from lack of expertise of the investigator or personnel shortages in team efforts. Money to buy equipment or chemicals and other expendibles is usually a conspicuous constraint. For this reason, scientists are constantly challenged to be as efficient as possible. This experimental laboratory recognizes that the ability to "get the most bang for the buck" is a desirable attribute for a scientist, science evaluator, science manager, or science consultant.

You will be given a personal supply of the following stock solutions:

1.	10	mL	2 M	tris (base)	[for **Extraction Cocktail** + **Assay Cocktail**]
2.	20	mL	1 N	HCl	[for **Extraction Cocktail** + **Assay Cocktail**]
3.	20	mL	100 mM	$MgCl_2$	[for **Extraction Cocktail** + **Assay Cocktail**]
4.	0.5	mL	10 mM	EDTA	[for **Extraction Cocktail**]
5.	2	mL	2 % (w/v)	BSA	[for **Assay Cocktail**]

You will have access to the following stock solutions sufficient to make a total of 160 mL of Assay Cocktail[1]:

1. NADPH, 10 mM
2. PGA, 100 mM
3. ATP, 100 mM
4. PGAK, 50 $\mu g\ mL^{-1}$

From the above solutions, you will make up 30 mL of **Extraction Cocktail.**[2] For each extract that you prepare, you will need 2 mL of Extraction Cocktail. You will make this cocktail only once, at the beginning of your experimental phase. It can be stored in a provided 8-oz. plastic bottle and frozen when not in use.

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[1]Be sure to read this entire section before beginning. Whereas all the extraction cocktail can be made at once and stored, the assay cocktail is made as a pre-mix that contains stable components. This pre-mix can be stored and aliquots can be amended to include the unstable components just before use.

[2]Add some H_2O to a mixing cylinder. Add tris (base) stock. Add the calculated amount of H^+. Mix well. Add 3^d component & mix well. ...add ith component.... Then, carefully add water to bring the solution to the desired volume. Mix well. Remove an aliquot to confirm the *p*H.

Extraction Cocktail
100 mM tris-Cl (*p*H 7.8)
1 mM $MgCl_2$
0.1 mM EDTA

With your stock solutions, you will also make up 160 mL **Assay Cocktail Pre-Mix** (ACPM). For each tube that you assay, you will need 3 mL of ACPM. You will make ACPM only once, at the beginning of your experimental phase. It also can be stored in a provided 8-oz. plastic bottle and frozen when not in use.

Assay-cocktail pre-mix
100 mM tris-Cl (*p*H 8.1)
10 mM $MgCl_2$
0.02 % (w/v) BSA

The complete assay cocktail contains:

1. Assay-cocktail pre-mix (100 mM tris-Cl (*p*H 8.1), 10 mM $MgCl_2$, 0.02%(w/v) BSA)
2. 0.5 μg mL^{-1} **P**hospho**g**lyceric **A**cid **K**inase (PGAK)
3. 75 μM NADPH
4. 1 mM ATP
5. 25 μL extract (nominal, 150 mg□2 mL^{-1}—3 leaf punches are ca. 150 mg)
6. ± 2 mM PGA

You will make the assay cocktail in the following way[3]:

1. Thaw ACPM, warm to room temperature, and mix well.
2. Remove the desired quantity to a mixing cylinder.
3. Have course personnel aliquot into the cylinder a specified amount of 50 μg mL^{-1} PGAK, mix well[4].
4. Have course personnel aliquot into the cylinder a specified amount of 10 mM NADPH; mix well.
5. Have course personnel aliquot into the cylinder a specified amount of 100 mM ATP.
6. Using a 3-mL volumetric pipette, deliver into each Spectronic 20™ [5] cuvette 3 mL of the ACPM that has been amended to include PGAK, NADPH, and ATP.
7. Add a specified amount of 100 mM PGA to specified cuvettes; mix and wait 2 min to allow PGA to be converted to 1,3-diPGA..
8. Add extract to the specified cuvettes; mix.
9. Immediately record optical density over a time course.

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[3]ACPM is stable at room temperature, to which it should be brought before the assay cocktail is prepared. After addition of PGAK, the reagent should be used not more than 30 minutes after preparation.
[4]The additions of PGAK, of ATP, and of NADPH cause only an inconsequential change in the volume and the concentration of components of the ACPM.
[5]Hereafter, simply "spec 20."

Very Important Note: You will be limited to a total of 80 μg of PGAK, 12 μmol NADPH, 160 μmol ATP, and 320 μmol PGA. This amount is sufficient for you to conduct 50 3-mL assays. By way of example, for a minimum experimental repetoire, you would run 4 x 3 mL assays in your validation experiments. Then you could set up the following tubes:

tube 1. -extract, -PGA
tube 2. -extract, +PGA
tube 3. -extract, -PGA
tube 4. -extract, +PGA

Then you would follow the ΔO.D./Δtime for tubes 1 & 2. The result would give you two control rates, (- extract, ± PGA). Then you could add extract A to tubes 1 & 2 and extract B to tubes 3 & 4. A spectrophotometric time course would then give you a control rate (+ extract, - PGA) and the full rate. Repetition of this experiment would require another 12 mL. Thus, the validation experiment (12 mL) plus repetition 1 (12 mL) plus repetition 2 (12 mL) would altogether consume 36 mL, or less than one-fourth of the allotted reagent. An important part of your overall evaluation will be an assessment of how much you were able to learn within the constraints of your resources, but you must be sure always to run the proper controls.