EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes

Preparing Karnovsky's Fixative From Scratch

Karnovsky's Fixative – A Little History

The original Karnovsky's fixative, developed by Dr. M. Karnovsky, was extremely hypertonic with 5% Glutaraldehyde and 4% Paraformaldehyde in 0.1M Cacodylate buffer. This fixative has been modified to lower aldehyde concentrations and even the use of Phosphate buffer. In fact, the name "Karnovsky's" is now a general term for any fixative which contains both Glutaraldehyde and Paraformaldehyde of various concentrations in a buffer of choice. The aldehydes in this fixative complement each other; paraformaldehyde is a small single carbon mono aldehyde which penetrates the tissue quickly and denatures proteins by reacting with the alpha amine group; glutaraldehyde is a larger 5 carbon dialdehyde which has a slower penetration rate, approximately 1 mm/hr, but crosslinks proteins with the two aldehyde groups stabilizing the structure better, reducing possible extraction during the subsequent processing steps.

Example: Making a Fixative

Want: 350 ml of 2.5% glutaraldehyde, 1.5% paraformaldehyde, in 0.1M of either sodium cacodylate, phosphate, or HEPESs buffer

Have: Sodium Cacodylate Trihydrate buffer, 100 g
Monobasic Sodium Phosphate buffer, 500 g
Dibasic Sodium Phosphate buffer, 500 g
HEPES buffer, 100 g

50% EM grade Glutaraldehyde, 10x10 ml
Paraformaldehyde - Granular, 500 g

EMS Catalog Supplies

Sodium Cacodylate Trihydrate buffer	#12300
Monobasic Sodium Phosphate buffer	#21190
Dibasic Sodium Phosphate buffer	#21180
HEPES buffer	#16782
50% EM grade Glutaraldehyde	#16320
Paraformaldehyde - Granular	#19208

HOW MUCH of EACH?

Buffers

Cacodylate: g = M x FW x Vol. (L) g = 0.1 x 214 x 0.350 **g = 7.49**

Phosphate: 1:2.5 (mono :dibasic) Volume 350 ml/3.5 (1 + 2.5) **= 100 ml**

Monobasic: g = M x FW x Vol. (L) g = 0.1 x 138 x 0.100 **g = 1.38**
Dibasic: g = M x FW x Vol. (L) g = 0.1 x 268 x 0.250 **g = 6.7**

HEPES: g = M x FW x Vol. (L) g = 0.1 x 238 x 0.350 **g = 8.33**

Fixatives

Paraformaldehyde: % x Volume 0.015 x 350 **g = 5.25**
Glutaraldehyde: C1 x V1 = C2 x V2 50% x ml? = 2.5% x 350 ml = **17.5 ml**

Steps for Making a Fixative

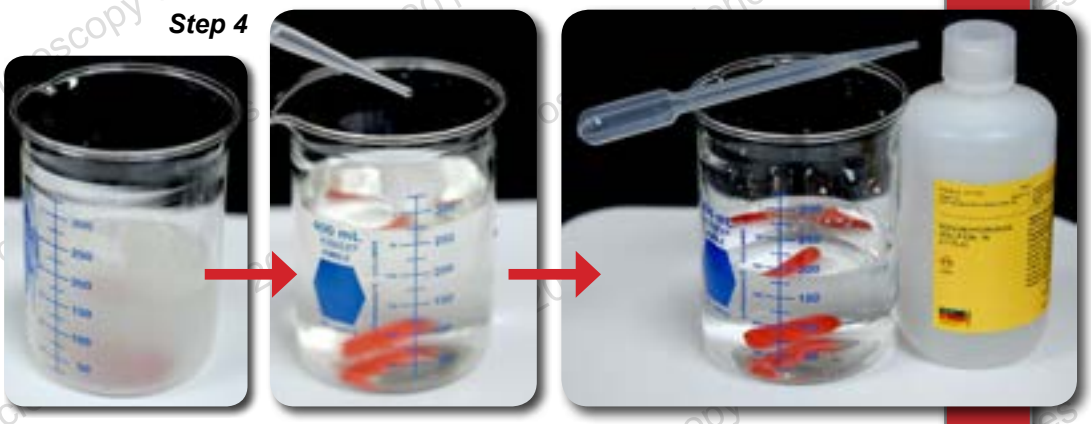
In a Fume Hood

1. In an appropriate container (500 ml Erlenmeyer flask or beaker), add about 300 ml of dH_2O and place on a stirring hot plate at 60C in a fume hood.
2. Measure out buffer material and, while stirring rapidly, add it to the heating water.

NOTE: *Paraformaldehyde goes into solution better in the presence of other ions and a high pH.*

3. When the water and buffer solution reach 60°C, *turn off the fume hood* and 1 gram of paraformaldehyde to the heated solution, wait until it goes into solution and continue with the next gram and follow this routine until all of the paraformaldehyde is in solution. BE SURE to turn the fume hood back on.

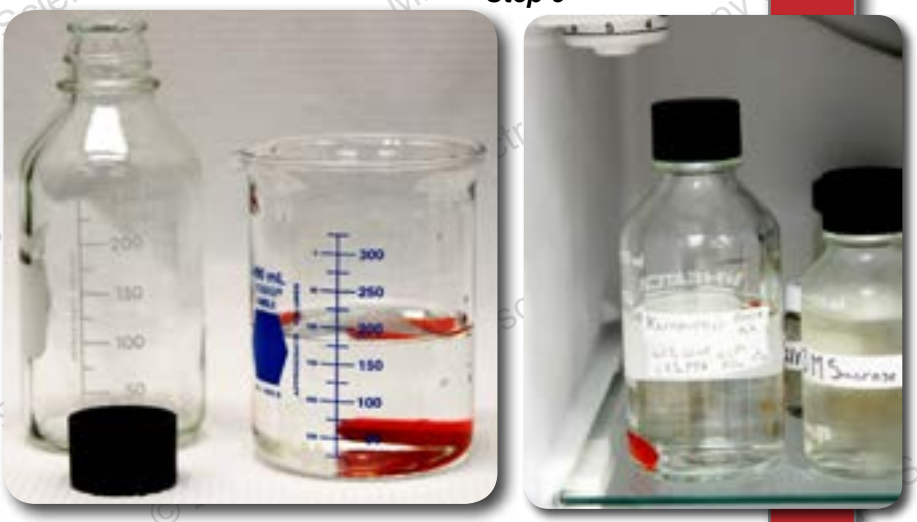
NOTE: *The fume hood was turned off to prevent the paraformaldehyde granules from dispersing in the air.*



4. The paraformaldehyde and buffer solution is now a milky white, to get everything into solution add 5M NaOH drops at a time until the solution clears.

5. Remove from heat, transfer to permanent storage container, cap it, and place in the refrigerator until it attains room temperature.

NOTE: *If Glutaraldehyde is added to the 60°C solution it will polymerize.*



6. When the solution is cool, measure and add the glutaraldehyde.

Step 6

7. Bring to final volume and adjust to desired pH.
8. Cover and store at 4°C.

Example

If you are using EM grade glutaraldehyde in sealed ampoules you should use the entire volume. In that case, determine the concentration you need and make the total volume of the solution so that all of the glutaraldehyde is used.

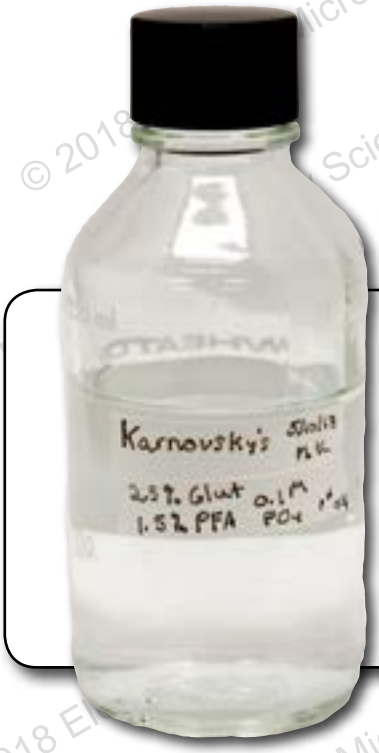
Using the above solution as an example that used 17.5 ml, and we want 2.5% glutaraldehyde We have 2 x 10 ml vial of 50% = 20 ml of 50% and want to use it all, then that formula will determine the final volume for the rest of the ingredients.

C1 x V1 = C2 x V2

We know the C1 (50%), V1 (20 ml), and the C2 (2.5%) and V2 is our unknown volume.

50% x 20 ml = 2.5% x V2 ml – V2 = 1000/2.5 = 400 ml

So now the total volume of Karnovsky's solution you are preparing is 400 not 350, go through the other equations and change the final volume. Make sure the phosphate buffer parts are changed to 400/3.5 = 114.3 so the volume of mono basic is 0.114L and di basic is 0.286L.



Label Your Solution

Remember, always clearly label your solution, stating:

- Date made
- Name of solution
- Concentration of solutes and/or vehicles used
- pH
- Your Initials

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