

## PROTOCOL:

# 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 1mm/hr, but crosslinks proteins with the two aldehyde groups stabilizing the structure better, reducing possible extraction during the subsequent processing steps.

### EXAMPLE:

**Want:** 350 ml of 2.5% Glutaraldehyde, 1.5% Paraformaldehyde, in 0.1M of either Sodium Cacodylate, Phosphate, or HEPES Buffer

**Have:** Sodium Cacodylate Trihydrate Buffer, 100g  
Monobasic Sodium Phosphate buffer, 500g  
Dibasic Sodium Phosphate Buffer, 500g  
HEPES buffer, 100g  
50% EM grade Glutaraldehyde, 10x10 ml  
Paraformaldehyde - Granular, 500 g

#### How much of each?

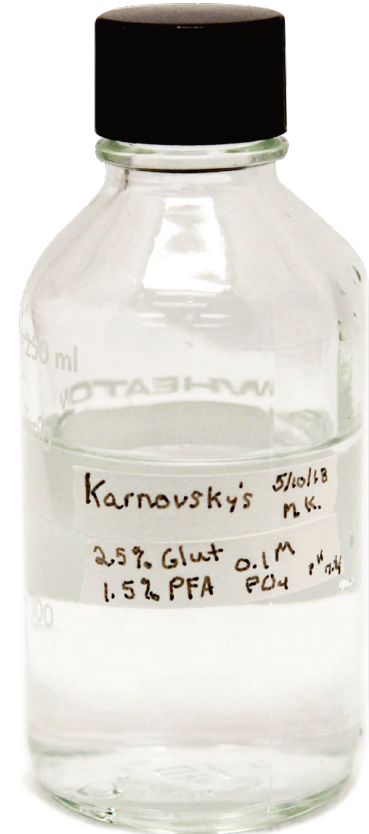
##### Buffers

<b>Cacodylate:</b>	$g = M \times FW \times Vol. (L)$	$g = 0.1 \times 214 \times 0.350$	<b>g = 7.49</b>
<b>Phosphate:</b>	1:2.5 (mono : dibasic) Volume 350 ml/3.5 (1 + 2.5)		<b>= 100 ml</b>
<b>Monobasic:</b>	$g = M \times FW \times Vol. (L)$	$g = 0.1 \times 138 \times 0.100$	<b>g = 1.38</b>
<b>Dibasic:</b>	$g = M \times FW \times Vol. (L)$	$g = 0.1 \times 268 \times 0.250$	<b>g = 6.7</b>
<b>HEPES:</b>	$g = M \times FW \times Vol. (L)$	$g = 0.1 \times 238 \times 0.350$	<b>g = 8.33</b>

##### Fixatives

<b>Paraformaldehyde:</b>	% x Volume	0.015 x 350	<b>g = 5.25</b>
<b>Glutaraldehyde:</b>	$C1 \times V1 = C2 \times V2$	50% x ml? = 2.5% x 350 ml	<b>= 17.5 ml</b>

Steps for Making Karnovsky's Fixative...see next page >>>



EMS Catalog supplies used **Cat. No.**

### Buffers

Sodium Cacodylate Trihydrate	<b>12300</b>
Monobasic Sodium Phosphate	<b>21190</b>
Dibasic Sodium Phosphate	<b>21180</b>
HEPES Buffer	<b>16782</b>

### Fixatives

50% EM-Grade Glutaraldehyde	<b>16320</b>
Paraformaldehyde — Granular	<b>19208</b>

**Steps for Making Karnovsky's Fixative**

**In a Fume Hood**

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



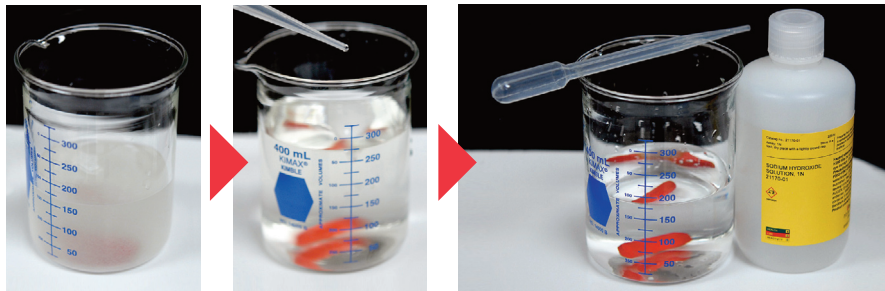
**NOTE:** Paraformaldehyde goes into solution better in the presence of other ions and high pH.

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.
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 solution on this page 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.

$$C1 \times V1 = C2 \times V2$$

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

$$50\% \times 20 \text{ ml} = 2.5\% \times V2 \text{ ml} - V2 = 1000/2.5 = 400 \text{ ml}$$

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

