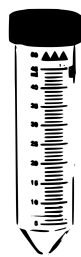


# Karnovsky's Fixative



FEB 13, 2023

## Karnovsky's Fixative

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### OPEN ACCESS

**Protocol Citation:** Jens Berndtsson 2023. Karnovsky's Fixative. **protocols.io** <https://protocols.io/view/karnovsky-39-s-fixative-ckiguubw>

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**Protocol status:** In development  
We are still developing and optimizing this protocol

**Created:** Dec 15, 2022

**Last Modified:** Feb 13, 2023

**PROTOCOL integer ID:**  
74024

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### ABSTRACT

Karnovsky's fixative is a standard fixative developed by M. J. Karnovsky and consist of: 2.5% glutaraldehyde, 2% formaldehyde, 0.02% sodium azide in 0.05 M Na-cacodylate buffer at pH 7.4.

#### FIXATION TIME:

- 1 hour minimum, 2-3 hours preferred, can be left overnight at 4 °C.
- For fixation of tissue, add to the fixative within 15 min of removal from patient.

### References

Karnovsky, M.J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron-microscopy. *Journal of Cell Biology*, 27, 137-138A.

## GUIDELINES

It is best to make 1L every time. Always two people – to check the measurements and so that one person can be closing tube lids while the other is pipetting to avoid the fixative evaporating.

## MATERIALS

⊗ Aqueous Glutaraldehyde EM Grade 25% 100ML **EMS Catalog #16210**

⊗ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710**

⊗ Sodium azide (99.5%) **BDH Chemicals Ltd Poole England Catalog #9115010C**

## SAFETY WARNINGS

! Use caution, work in a fume hood, wear gloves, lab coat, and goggles.

⊗ Aqueous Glutaraldehyde EM Grade 25% 100ML **EMS Catalog #16210**

H330 Fatal if inhaled.

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H314 Causes severe skin burns and eye damage.

H318 Causes serious eye damage.

H302 Harmful if swallowed.

H317 May cause an allergic skin reaction.

H335 May cause respiratory irritation.

⊗ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710**

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled

H315 Causes skin irritation.

H319 Causes serious eye irritation

H227 Combustible liquid.

⊗ Sodium azide (99.5%) **BDH Chemicals Ltd Poole England Catalog #9115010C**

H300 + H310 + H330 Fatal if swallowed, in contact with skin or if inhaled.

H373 May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed.

H410 Very toxic to aquatic life with long lasting effects.

## BEFORE START INSTRUCTIONS

Book a fume hood and label the aliquot tubes.

### 1000 ml

#### 1 Mix the following in a 1000 ml beaker:

- 100 mL of Aqueous Glutaraldehyde EM Grade 25% 100ML EMS Catalog #16210
- 125 mL of  
PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710
- 0.2 g of  
Sodium azide (99.5%) BDH Chemicals Ltd Poole England Catalog #9115010C
- 250 mL of [M] 200 millimolar (mM) Na-cacodylate buffer
- 525 mL of Milli-Q water Contributed by users

#### Aliquote the mixture in:

- 20 x 5 mL - 15 ml falcon tube labelled K<sub>5</sub>
- 30 x 10 mL - 15 ml falcon tube labelled K<sub>10</sub>
- 30 x 20 mL - 50 ml falcon tube labelled K<sub>20</sub>

*Dispose of the pipettes used for aliquoting in a hazardous waste bin.*

#### Storage

Store long term (6-12 months) at -20 °C or short term (1-2 weeks) at 4 °C

#### Clean up:




Rinse the used 1000 ml beaker twice with 100 ml of water, disposing it in a liquid hazardous waste-jar for fixatives before putting the beaker in the dishwasher. Treat spillage as dangerous and discard any contaminated safety paper in a hazardous waste bin. Spills outside of safety papers should be cleaned up with paper and water which should then be discarded in a hazardous waste bin.

### 500 ml

#### 2 Mix the following in a 500 ml beaker:

- 50 mL of Aqueous Glutaraldehyde EM Grade 25% 100ML EMS Catalog #16210
- 62.5 mL of  
PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710
- 0.1 g of  
Sodium azide (99.5%) BDH Chemicals Ltd Poole England Catalog #9115010C
- 125 mL of [M] 200 millimolar (mM) Na-cacodylate buffer
- 262.5 mL of Milli-Q water Contributed by users

**Aliquote the mixture in:**

- 10 x  5 mL - 15 ml falcon tube labelled K<sub>5</sub>
- 15 x  10 mL - 15 ml falcon tube labelled K<sub>10</sub>
- 15 x  20 mL - 50 ml falcon tube labelled K<sub>20</sub>

*Dispose of the pipettes used for aliquoting in a hazardous waste bin.*

**Storage**











Store long term (6-12 months) at  -20 °C or short term (1-2 weeks) at  4 °C

**Clean up:**




Rinse the used 500 ml beaker twice with 100 ml of water, disposing it in a liquid hazardous waste-jar for fixatives before putting the beaker in the dishwasher. Treat spillage as dangerous and discard any contaminated safety paper in a hazardous waste bin. Spills outside of safety papers should be cleaned up with paper and water which should then be discarded in a hazardous waste bin.

## 200 ml

**3 Mix the following in a 500 ml beaker:**

-  20 mL of  Aqueous Glutaraldehyde EM Grade 25% 100ML EMS Catalog #16210
-  25 mL of  PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710
-  0.04 g of  Sodium azide (99.5%) BDH Chemicals Ltd Poole England Catalog #9115010C
-  50 mL of  200 millimolar (mM) Na-cacodylate buffer
-  105 mL of  Milli-Q water Contributed by users

**Aliquote the mixture in:**

- 4 x  5 mL - 15 ml falcon tube labelled K<sub>5</sub>
- 6 x  10 mL - 15 ml falcon tube labelled K<sub>10</sub>
- 6 x  20 mL - 50 ml falcon tube labelled K<sub>20</sub>

*Dispose of the pipettes used for aliquoting in a hazardous waste bin.*

**Storage**

Store long term (6-12 months) at  -20 °C or short term (1-2 weeks) at  4 °C

**Clean up:**

Rinse the used 500 ml beaker twice with 100 ml of water, disposing it in a liquid hazardous waste-jar for fixatives before putting the beaker in the dishwasher. Treat spillage as dangerous and discard any contaminated safety paper in a hazardous waste bin. Spills outside of safety papers should be cleaned up with paper and water which should then be discarded in a hazardous waste bin.

bin.

## 100 ml

### 4 Mix the following in a 100 ml beaker:

- 10 mL of Aqueous Glutaraldehyde EM Grade 25% 100ML EMS Catalog #16210
- 12.5 mL of  
PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710
- 0.02 g of  
Sodium azide (99.5%) BDH Chemicals Ltd Poole England Catalog #9115010C
- 25 mL of 200 millimolar (mM) Na-cacodylate buffer
- 52.5 mL of Milli-Q water Contributed by users

### Aliquote the mixture in:

- 2 x 5 mL - 15 ml falcon tube labelled K<sub>5</sub>
- 3 x 10 mL - 15 ml falcon tube labelled K<sub>10</sub>
- 3 x 20 mL - 50 ml falcon tube labelled K<sub>20</sub>

*Dispose of the pipettes used for aliquoting in a hazardous waste bin.*

### Storage

Store long term (6-12 months) at -20 °C or short term (1-2 weeks) at 4 °C

### Clean up:

Rinse the used 100 ml beaker twice with 20 ml of water, disposing it in a liquid hazardous waste-jar for fixatives before putting the beaker in the dishwasher. Treat spillage as dangerous and discard any contaminated safety paper in a hazardous waste bin. Spills outside of safety papers should be cleaned up with paper and water which should then be discarded in a hazardous waste bin.