

Glutaraldehyde fixation

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Working



ABSTRACT

Fixation of adherent mammalian cells for AFM imaging

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Prepare adherent cell culture on glass coverslips

MATERIALS

NAME	CATALOG #	VENDOR
Glutaraldehyde, 25% solution	GC3870.SIZE.500ml	Bio Basic Inc.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
PBS		

SAFETY WARNINGS

Glutaraldehyde requires a high degree of caution, even at low concentration! Carefully read SDS before use!

Classification according to Regulation (EC) No 1272/2008

Acute toxicity, Oral (Category 4), H302 Acute toxicity, Inhalation (Category 4), H332 Skin corrosion (Sub-category 1B), H314 Serious eye damage (Category 1), H318 Respiratory sensitisation (Category 1), H334 Skin sensitisation (Category 1), H317 Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335 Short-term (acute) aquatic hazard (Category 1), H400 Long-term (chronic) aquatic hazard (Category 2), H411

Refer to SDS for details

Side effects include skin irritation.^[4] If exposed to large amounts, nausea, headache, and shortness of breath may occur.^[3] [Protective equipment](#) is recommended when used.^[3] Glutaraldehyde is effective against a range of [microorganisms](#) including [spores](#).^{[3][6]} As a strong sterilant, glutaraldehyde is [toxic](#) and a strong irritant.^[15] There is no strong evidence of [carcinogenic](#) activity.^[16] Some occupations that work with this chemical have an increased risk of some cancers.^[16]

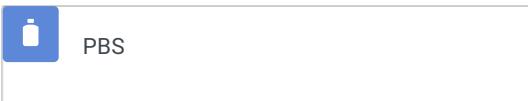
BEFORE STARTING

Ensure proper disposal container is available in the area

Perform entire procedure in ventilated fumehood, disinfect workspace and tools with ethanol

Use gloves and eye protection (fumehood should be sufficient to prevent inhalation so a mask is not required)

- 1 Rinse cells 3 times with appropriate buffer, such as PBS or Ringer's

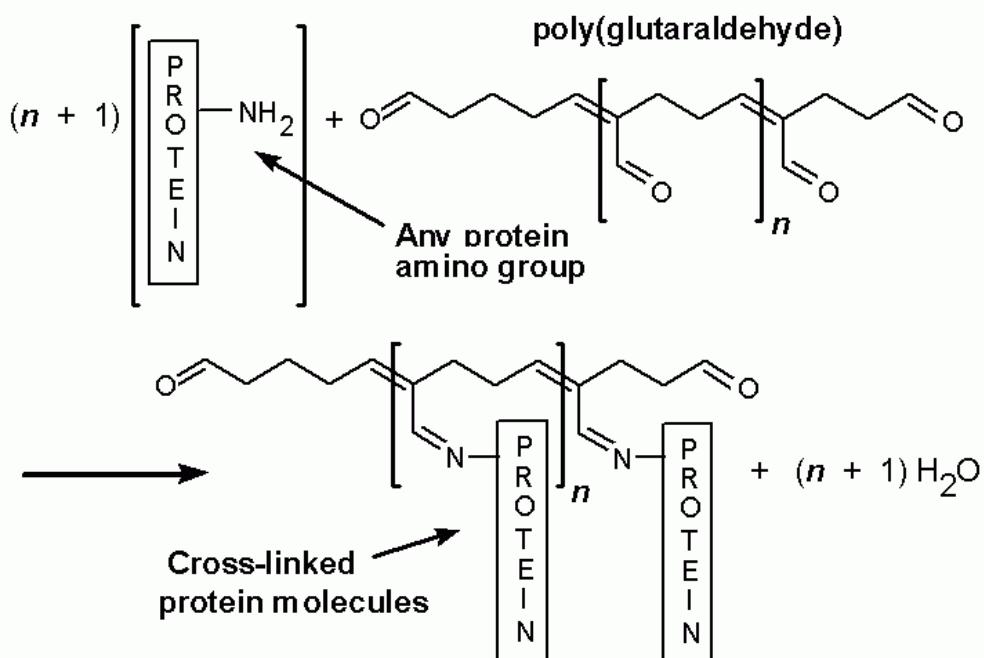


- 2 Prepare [M]2 Mass/Volume Percent concentration glutaraldehyde in KMgH buffer (or other buffer used for your application).

Pipette just enough of the dilution on top of the rinsed cells to cover the area. Let sit for 00:20:00



Glutaraldehyde chemical formula



<http://publish.uwo.ca/~jkiernan/formglut.htm>

- 3 Rinse at least 3 times with buffer, properly disposing of the diluted glutaraldehyde.

EXPECTED RESULT

Glutaraldehyde is appropriate for whole and unroofed cell fixation, so up to 100% should be fixed with decent crosslinking. For thicker samples use an addition of paraformaldehyde

SAFETY INFORMATION

Use sodium hydroxide for deactivation if necessary

http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0936/0901b80380936cd5.pdf?filepath=microbial/pdfs/noreg/253-03304.doc&fromPage=GetDoc



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