Fixing Cells with Paraformaldehyde (PFA)

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Abstract

References:

Becton Dickinson Immunocytometry Systems Source Book (1989) 2.10

Lanier, L.L., and Warner, N.L. (1981) Paraformaldehyde Fixation of Hematopoietic Cells for Quantitative Flow Cytometry (FACS) Analysis. Journal of Immunological Methods 47, 25

C.A. Williams, M.W. Chase, Stabilizes cell membranes and preserves cell morphology. *Methods Immunol. Immunochem.* New York **5**, (1976)

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Guidelines

Needed:

1. 16% Formaldehyde
2. PBS
3. Ice

Working Dilutions:

Thawed aliquots are stable at 4º C for up to 2 weeks.

Dilute 1 part 2% PFA to 3 parts cells in PBS (eg 60 µl + 180 µl to yield 0.5% final concentration).

Materials

16% Formaldehyde Solution (10 ml ampoules) 0173 by Canemco & Marivac

Protocol

Preparation of Paraformaldehyde from Powder

Step 1.

Prepare PFA from powder

PROTOCOL

. Paraformaldehyde (PFA) from Powder

CONTACT: VERVE Team

Prepare PFA From Powder

Step 1.1.
Allow paraformaldehyde (PFA) powder to come to room temperature

NOTES
VERVE Team 10 Aug 2015
(Stored in refrigerator)

Prepare PFA From Powder

Step 1.2.
Weigh 10.0 g PFA in fume hood.

AMOUNT
10 g Additional info:

REAGENTS
Paraformaldehyde Powder (PFA) P6148 by Contributed by users

Prepare PFA From Powder

Step 1.3.
Flush container with Argon or Nitrogen to prevent air decomposition of p-formaldehyde.

Prepare PFA From Powder

Step 1.4.
Dissolve in 475 ml distilled H_2O in 60-70º C water bath on hot plate in fume hood.

AMOUNT
475 ml Additional info:

NOTES
VERVE Team 22 Jun 2015
**Do not allow water bath to go over 70ºC (formaldehyde will vaporize!).

Prepare PFA From Powder

Step 1.5.
While disolving, label 100 X 4 ml and 7 X 12 ml tubes (or other combinations of useful aliquots) with the concentration and date.

Prepare PFA From Powder

Step 1.6.
After 1 hr add 1 or 2 drops of 5M NaOH.

DURATION
01:00:00

NOTES
VERVE Team 22 Jun 2015
Cloudy suspension will then turn clear.

Prepare PFA From Powder

Step 1.7.
Allow to cool at room temperature (@2hours)

DURATION
01:00:00

Prepare PFA From Powder

Step 1.8.
Add 25 ml 20X PBS (See Recipie)

AMOUNT
25 ml Additional info:

Step 1.9.
Adjust pH to 7.3.

Step 1.10.
Filter, aliquot into tubes and freeze.

NOTES
VERVE Team 22 Jun 2015
Aliquots good for at least 5 years.
Thawed aliquots are stable at 4º C for up to 2 weeks.

Prepare PFA From Powder

Step 1.11.
Dilute 1 part 2% PFA to 3 parts cells in PBS (eg 60 µl + 180 µl to yield 0.5% final concentration).

Preparation from a Stock Solution

Step 2.
Dilute with PBS.

NOTES
VERVE Team 22 Jun 2015
Dilute only the amount of PFA you will need per experiment to 4% PFA from the 16% stock

Preparation from a Stock Solution

Step 3.
Store the undiluted stock at -20°C until needed.

NOTES
VERVE Team 22 Jun 2015
Open stocks should only be kept for one month

Preparation from a Stock Solution

Step 4.
Add and equal volume of the 4% stock to samples to end up with a final concentration of at most 2% PFA.

NOTES
VERVE Team 10 Aug 2015
Fixation can be done from 0.5-2%.

Preparation from a Stock Solution

Step 5.
Fix cells on ice for 15-30 minutes on ice,

DURATION
00:30:00

NOTES
VERVE Team 10 Aug 2015
Fixation can be done from 0.5-2%.

Preparation from a Stock Solution

Step 6.
Wash 2x with PBS

NOTES
VERVE Team 10 Aug 2015
Fixation can be done from 0.5-2%.

Warnings

Samples should never be left in PFA overnight. This dramatically increases the amount of autofluorescence your samples. Diluted PFA (2-4% solutions) are only good for 1 week, therefore always date you PFA.