# Paraformaldehyde (PFA) from Powder

# Faculty of Medicine Flow Cytometry Facility, U. of Toronto

#### **Abstract**

This is a guide to prepare PFA from powder. This is useful for PFA Fixation.

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#### **Guidelines**

# **Working Dilutions:**

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

Dilute 1 part 2% PFA to 3 parts cells in PBS (eq  $60 \mu + 180 \mu$  to yield 0.5% final concentration).

# **Protocol**

#### Prepare PFA From Powder

#### Step 1.

Allow paraformaldehyde (PFA) powder to come to room temperature

#### NOTES

VERVE Team 10 Aug 2015

(Stored in refrigerator)

### Prepare PFA From Powder

#### Step 2.

Weigh 10.0 g PFA in fume hood.



10 g Additional info:



**REAGENTS** 

✓ Paraformaldehyde Powder (PFA) P6148 by Contributed by users

#### Prepare PFA From Powder

#### Step 3.

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

#### Prepare PFA From Powder

Dissolve in 475 ml distilled H<sub>2</sub>O in 60-70° C water bath on hot plate in fume hood.



475 ml Additional info:

#### NOTES

VERVE Team 22 Jun 2015

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\*\*Do not allow water bath to go over 70°C (formaldehyde will vaporize!).

#### Prepare PFA From Powder

# Step 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 6.

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

**O DURATION** 

01:00:00

#### NOTES

# VERVE Team 22 Jun 2015

Cloudy suspension will then turn clear.

# Prepare PFA From Powder

# Step 7.

Allow to cool at room temperature (@2hours)

**O DURATION** 

01:00:00

#### Prepare PFA From Powder

#### Step 8.

Add 25 ml 20X PBS (See Recipie)

**■** AMOUNT

25 ml Additional info:

# Step 9.

Adjust pH to 7.3.

# Step 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  $4^{\circ}$  C for up to 2 weeks.

#### Prepare PFA From Powder

# **Step 11.**

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