

## PARAFORMALDEHYDE (PFA) FOR ADULT DROSOPHILA

*Concentrated PFA is a hazardous chemical. Please follow all appropriate lab safety practices for dealing with hazardous chemicals. Be self-informed on the hazards of PFA.*



- Always wear gloves when handling PFA.
- The 20% PFA stock solution should only be opened in the hood.
- If PFA comes into contact with your skin or eyes, immediately flush the affected area with water.
- Containers and pipette tips that have come into contact with the 20% PFA should be discarded into the red sharps container in the hood.
- MSDS available at <http://www.emsdiasum.com/microscopy/technical/msds/15713.pdf>

*Harmful if swallowed • harmful when in contact with skin • harmful if inhaled • causes skin irritation  
• causes serious eye irritation • may cause an allergic skin reaction • may cause respiratory irritation  
• suspected of causing cancer*

1. Determine the number of tubes of diluted fix you will need to make.
2. Determine the concentration of fix you will make: 1.2% PFA, 2% PFA or 4% PFA
3. Use the dilution tables to determine the volumes of 20% PFA and diluent (S2 or PBS) that will be combined to make the desired concentration of PFA.
  - Diluents:
    - Primary fixation following dissection: Schneider's Insect Medium 2 (S2)
    - Pre-embedding fixation: PBS-1X
4. Measure out the appropriate volume of 20% PFA and transfer it to a conical tube.
  - 20% PFA stock aliquots are stored at RT in Eppendorf 2 mL **natural tubes** protected from light.
5. Use a repeater or serological pipet to measure the correct amount of diluent and add it to the conical tube.
  - Use the pipet (5 mL, 10 mL or 25 mL) that is closest to the volume you need to measure.
  - Dispose of the serological pipet in the **sharps container**.
6. Seal the conical tube tightly and vortex.
7. Dispense aliquots of approximately 1.90 mL of the diluted PFA into Protein LoBind tubes.
8. Store the diluted PFA according to your project requirements. Always protected the PFA from light.

### Reagents and Supplies

- Natural Microcentrifuge Tubes - 2 mL. Eppendorf. # 022363352 (for storing 20% PFA aliquots)
- PFA – Paraformaldehyde. 20% PFA. Electron Microscopy Sciences. # 15713-S
- Protein LoBind Microcentrifuge Tubes - 2 mL. Eppendorf. # 022431102. (for diluted PFA ≤4%)

PFA Dilution Charts

**1.2% PFA in S2**

Adult CNS or Brains

# Tubes	Total Volume (mL)	20% PFA (mL)	S2 (mL)
1	2.00	0.12	1.88
2	4.00	0.24	3.76
3	6.00	0.36	5.64
4	8.00	0.48	7.52
5	10.00	0.60	9.40
6	12.00	0.72	11.28
7	14.00	0.84	13.16
8	16.00	0.96	15.04
9	18.00	1.08	16.92
10	20.00	1.20	18.80
11	22.00	1.32	20.68
12	24.00	1.44	22.56
13	26.00	1.56	24.44
14	28.00	1.68	26.32
15	30.00	1.80	28.20
16	32.00	1.92	30.08
17	34.00	2.04	31.96
18	36.00	2.16	33.84
19	38.00	2.28	35.72
20	40.00	2.40	37.60
21	42.00	2.52	39.48
22	44.00	2.64	41.36
23	46.00	2.76	43.24
24	48.00	2.88	45.12
25	50.00	3.00	47.00
26	52.00	3.12	48.88
27	54.00	3.24	50.76
28	56.00	3.36	52.64
29	58.00	3.48	54.52
30	60.00	3.60	56.40

**2% PFA in S2**

Adult CNS or Brains

# Tubes	Total Volume (mL)	20% PFA (mL)	S2 (mL)
1	2.00	0.20	1.80
2	4.00	0.40	3.60
3	6.00	0.60	5.40
4	8.00	0.80	7.20
5	10.00	1.00	9.00
6	12.00	1.20	10.80
7	14.00	1.40	12.60
8	16.00	1.60	14.40
9	18.00	1.80	16.20
10	20.00	2.00	18.00
11	22.00	2.20	19.80
12	24.00	2.40	21.60
13	26.00	2.60	23.40
14	28.00	2.80	25.20
15	30.00	3.00	27.00
16	32.00	3.20	28.80
17	34.00	3.40	30.60
18	36.00	3.60	32.40
19	38.00	3.80	34.20
20	40.00	4.00	36.00
21	42.00	4.20	37.80
22	44.00	4.40	39.60
23	46.00	4.60	41.40
24	48.00	4.80	43.20
25	50.00	5.00	45.00
26	52.00	5.20	46.80
27	54.00	5.40	48.60
28	56.00	5.60	50.40
29	58.00	5.80	52.20
30	60.00	6.00	54.00

**4% PFA in 1xPBS**

Adult CNS or Brains

# Tubes	Total Volume (mL)	20% PFA (mL)	1xPBS (mL)
1	2.00	0.40	1.60
2	4.00	0.80	3.20
3	6.00	1.20	4.80
4	8.00	1.60	6.40
5	10.00	2.00	8.00
6	12.00	2.40	9.60
7	14.00	2.80	11.20
8	16.00	3.20	12.80
9	18.00	3.60	14.40
10	20.00	4.00	16.00
11	22.00	4.40	17.60
12	24.00	4.80	19.20
13	26.00	5.20	20.80
14	28.00	5.60	22.40
15	30.00	6.00	24.00
16	32.00	6.40	25.60
17	34.00	6.80	27.20
18	36.00	7.20	28.80
19	38.00	7.60	30.40
20	40.00	8.00	32.00
21	42.00	8.40	33.60
22	44.00	8.80	35.20
23	46.00	9.20	36.80
24	48.00	9.60	38.40
25	50.00	10.00	40.00
26	52.00	10.40	41.60
27	54.00	10.80	43.20
28	56.00	11.20	44.80
29	58.00	11.60	46.40
30	60.00	12.00	48.00