Fixative Protocols and Recipes
(from IHC world)

Formalin Solution (10%, unbuffered):
Formaldehyde (37-40%) ---------- 10 ml
Distilled water --------------------- 90 ml
Mix well.

Formalin Solution (10%, buffered neutral):
Formaldehyde (37-40%) ---------- 100 ml
Distilled water --------------------- 900 ml
NaH2PO4 -------------------------- 4.0 g
Na2HPO4 (anhydrous) --------------- 6.5 g
Mix to dissolve.

Formalin-Acetic Acid Solution:
Formaldehyde (37-40%) ---------- 10 ml
Distilled water --------------------- 90 ml
Glacial acetic acid ---------------- 5 ml
Mix well.

Formalin-Alcoholic Solution:
Formaldehyde (37-40%) ---------- 10 ml
Ethanol (80%) -------------------- 90 ml
Mix well.

Bouin Solution:
Picric acid (saturated) -------------- 75 ml
Formaldehyde (37-40%) -------------- 25 ml
Glacial acetic acid ---------------- 5 ml
Mix well. For routine surgical material, especially for preserving soft and delicate structures such as brain tissues.

Carnoy Solution:
Ethanol (absolute) -------------- 60 ml
Chloroform ---------------------- 30 ml
Glacial acetic acid -------------- 10 ml
Mix well.
Note: Used for fixation of DNA, RNA, Nissl granules and glycogen.

Helly Solution:
Mercuric chloride ------------ 5 g
Potassium dichromate --------- 2.5 g
Distilled water ------------- 100 ml
Heat - cool - filter in brown bottle. Add 5 ml formaldehyde just before use.
Wash in running tap water for 24 hours before dehydraation.
Note: Used for blood forming organs such as bone marrow, liver and spleen.

Susa Solution:

Stock Solution A:
Mercuric chloride -------------- 4.5 g
Sodium chloride -------------- 0.5 g
Trichloracetic acid ------------ 2 g
Distilled water ------------- 80 ml

Stock Solution B:
Glacial acetic acid ------------ 4 ml
Formaldehyde (37-40%) ------ 20 ml
Mix Solution A and B. For hard tissues such as inner ear with excellent penetration and little shrinkage.

Zenker Solution:
Mercuric chloride ------------ 5 g
Potassium dichromate --------- 2.5 g
Distilled water ------------- 100 ml
Heat - cool - filter in brown bottle. Add 5 ml of glacial acetic acid just before use. Wash fixed tissue in running tap water for 24 hours before dehydration. Never use metal forceps or metal container when handling tissues fixed in Zenker Solution.
Note: Used for bloody specimens such as spleen as well as connective tissues.

Zinc Fixative:

0.1M Tris Buffer, pH 7.4:
Tris Base --------------------- 12.1 g (TRIZMA)
1N HCL ---------------------- 81.5 ml
Distilled water ------------- 900 ml

Zinc Fixative:
Calcium Acetate ------------- 0.5 g
Zinc Acetate --------------- 5.0 g
Zinc Chloride --------------- 5.0 g
0.1M Tris Buffer made above ----- 1000 ml
Mix to dissolve. The final pH will be approximately 6.5-7.0. Do not readjust the pH, as this will cause the zinc to come out of solution. Store Zinc Fixative at room temperature. Fix tissues for 24 to 48 hours.
**4% Paraformaldehyde in 0.1M Phosphate Buffer**

0.2M Phosphate Buffer (PB), pH7.4:
- Na₂HPO₄ --------------- 21.8 g
- NaH₂PO₄ --------------- 6.4 g
- Distilled water --------- 1000 ml

0.1M Phosphate Buffer, pH7.4:
- 0.2M PB --------------- 500 ml
- Distilled water --------- 500 ml

**4% Paraformaldehyde in 0.1M Phosphate Buffer:**
- Paraformaldehyde --------- 40 g
- 0.1M Phosphate buffer --------- 1000 ml

Heat to 60-65 ºC while stirring. Add a few drops of 1N NaOH until solution clear. Continue to stir to dissolve. Cool the solution and filter.

Note: This solution is often used for animal perfusion.

**4% Paraformaldehyde-1% Glutaraldehyde in 0.1M Phosphate Buffer**

0.2M Phosphate Buffer (PB), pH7.4:
- Na₂HPO₄ --------------- 21.8 g
- NaH₂PO₄ --------------- 6.4 g
- Distilled water --------- 1000 ml

0.1M Phosphate Buffer, pH7.4:
- 0.2M PB --------------- 500 ml
- Distilled water --------- 500 ml

**4% Paraformaldehyde-1% Glutaraldehyde in 0.1M PB:**
- Paraformaldehyde --------- 40 g
- 0.1M Phosphate buffer --------- 1000 ml

Heat to 60-65 ºC while stirring. Add a few drops of 1N NaOH until solution clear. Continue to stir to dissolve. Cool the solution and filter. Add 20 ml of 50% glutaraldehyde and mix well.

Note: This solution is often used for animal perfusion, especially for electron microscopy.