Some Immuno-EM Solutions:
Adapted from protocols used in Utrecht, NL.

PHEM buffer:
0.4M PHEM buffer (this is a 4x stock solution!) is an excellent buffer for immuno-EM fixation.

<table>
<thead>
<tr>
<th></th>
<th>[conc.]:</th>
<th>MW:</th>
<th>for 500 mL:</th>
<th>for 100 mL:</th>
<th>for 50 mL:</th>
</tr>
</thead>
<tbody>
<tr>
<td>pipes</td>
<td>240 mM</td>
<td>302.40</td>
<td>36.2 g</td>
<td>7.26 g</td>
<td>3.63 g</td>
</tr>
<tr>
<td>hepes</td>
<td>100 mM</td>
<td>238.30</td>
<td>11.9 g</td>
<td>2.38 g</td>
<td>1.19 g</td>
</tr>
<tr>
<td>MgCl₂.6H₂O</td>
<td>8 mM</td>
<td>203.30</td>
<td>0.81 g</td>
<td>0.16 g</td>
<td>0.081 g</td>
</tr>
<tr>
<td>EGTA</td>
<td>40 mM</td>
<td>380.35</td>
<td>7.6 g</td>
<td>1.52 g</td>
<td>0.76 g</td>
</tr>
</tbody>
</table>

pH = 6.9

Formvar:
1.1% formvar for grid coating.

Rinse all glassware with acetone first, then with chloroform before drying out in a drying stove. Place 1.1 grams of formvar (formvar 15/95E, for instance Sigma F-6146) in an erlenmeyer flask with glass stopper. Add chloroform to 3/4th full, stir until dissolved and top up to 100 mL mark. This stock solution will keep for a while (up to a few weeks) as long as it is well sealed and kept in the dark.

10% Gelatin:
Dissolve 10 grams of gelatin powder (sometimes any generic supermarket brand is better than a pure grade from Fisher or Sigma) in 100 mL 0.1 M phosphate buffer that is at 60°C. Add 200 µL 10% azide and centrifuge to spin out any contaminants. Divide into smaller tubes and keep refrigerated.

10% BSA:
Place 10 grams of BSA in a beaker and add 100 mL aqua dest. (or 0.1 M phosphate buffer if you prefer). Cover and let stir slowly (to avoid foaming) overnight in the cold room. Bring the pH up to 7.4 with 1 N NaOH. Add 200 µL 10% sodium azide and centrifuge to spin out any contaminants. Divide into smaller tubes and keep refrigerated.
**Sucrose:**
2.3M Sucrose in phosphate buffer

Take a glass beaker, fill with 100 mL 0.1 M phosphate buffer pH 7.4, add a stir bar and mark the meniscus. Weigh out 80 grams of sucrose (MW 342.3) into the empty beaker and fill to the mark with phosphate buffer.

**Methyl Cellulose:**
(15 cp, as is still on our shelf, or Sigma M-6385, which is 25 centipoises)

For 100 mL:

Bring 98 mL aqua dest. to a temperature of 90°C (cover with glass dish to avoid excess evaporation), remove from heat and add 2 grams of methyl cellulose; stir. When well mixed, place in ice and stir occasionally. Keep covered. When the solution reaches a temperature of 10°C seal with parafilm and leave stirring overnight in the cold room. Turn off stirring the next morning and let the mixture “ripen” for 3 more days before centrifuging. Use the SW28 rotor, divide the MC into 3 centrifuge tubes and place into holders. Fill 1 tube with water, and leave 2 of the 6 tubes empty. Balance holders with tubes and make sure the empty tubes will be across from each other in the rotor. Centrifuge for 90 minutes at 25,000 rpm (± 100,000 G).

**MC-UA embedding mix:**

Substitute 10% of the volume of a 2% methyl cellulose solution with 4% uranyl acetate for an end solution of 1.8% MC / 0.4% UA which is used for the final embedding of ultrathin cryosections on grids.

**Neutral Uranyl Acetate (not commonly used much anymore for immuno-EM):**
(Alpha-emitter – light sensitive)

2% neutral uranyl acetate in aqua dest.
Make up 4% uranyl acetate in aqua dest. (2 g in 50 mL).
Make up 0.3 M Oxalic acid (1.9 g in 50 mL).

Take 4% Uranyl acetate and 0.3 M Oxalic acid and mix 1:1
This will give 2% UA in 0.15 M Oxalic acid.

Add 25% Ammonium Hydroxide drop by drop to a pH of 7, or a little over. Do this under constant stirring (in fume hood!) and monitor the pH with a stick. If you go over 7.5 or 8 then a precipitate forms and you can start all over again. Note that if the ammonium is too old, or has been opened too many times, the concentration of ammonium changes with the end result that you have to add too much “ammonium” to your solution. This will give you a neutral UA with less contrasting ability than a fresher ammonium solution.