PBS 10x

**Materials and equipment**

1. Erlenmeyer, 2.5 L
2. Graduated cylinder
3. pH meter
4. Stirring bar

**Reagents**

1. H2O
2. KCL
3. Na2HPO4.2H2O
4. NaCl
5. NaH2PO4.1H2O

**Procedure:**

1. Add to 1,500 mL of distilled water 5.75 g of NaH2PO4.1H2O and 36 g of Na2HPO4.2H2O.
2. Let dissolve completely before adding 200 g of NaCl and 5 g of KCl.
3. Stir, remove stirrer bar when everything is dissolved and add distilled water till 2,500 mL. Check if pH7.4.
4. Store at RT.

Phosphate buffer 0.2 M, pH 7.4, 100 mL

**Materials and equipment**

1. Erlenmeyer flasks (3), 100 mL
2. Graduated cylinders (2)
3. Magnetic stirrer
4. pH meter
5. Stirring bars (3)

**Reagents**

1. NaH2PO4. 1H2O
2. Na2HPO4. 2H2O
3. H2O

**Procedure**

1. Stock A: 0.2 M NaH2PO4.1H2O
	1. Add 2.76 g in 70 mL of distilled water, stir.
	2. Add distilled water till 100 mL.
	3. Store at room temperature.
2. Stock B: 0.2 M Na2HPO4. 2H2O
	1. Add 3.56 g in 70 mL of distilled water and stir. Add distilled water to a final volume of100 mL.
	2. Store at room temperature.
3. 0.2 M Phosphate buffer, pH 7.4

NOTE: Before use

* 1. Add 81 mL of stock B to 19 mL of stock A and stir.
	2. Check the pH.

PHEM 0.02 M buffer, pH 6.9

**Materials and equipment**

1. Erlenmeyer, 100 mL
2. Graduated cylinder
3. Magnetic stirrer
4. pH meter
5. Stirring bar

**Reagents**

1. EGTA [Merck 324626]
2. Hepes [Merck 391340]
3. MgCl2. 6H2O
4. NaOH 1 M (0.4 g/10 mL distilled water)
5. Pipes (Piperazin-1,4-bis (2 ethansulfonsäure) [Merck 110220]

**Procedure**

1. Add 3 pellets of NaOH to 75 mL of dH2O and stir.
2. Add 3.63 g of Pipes (120 mM); stir at RT till the solution is clear. Check the pH (~pH 7).
3. Add 1.19 g of Hepes (50 mM) and stir.
4. Add 0.08 g of MgCl2 (4 mM) and stir.
5. Add 0.76 g of EGTA (20 mM) and stir.
6. Adjust the pH to 6.9 with 1 M NaOH. Add dH2O to a final volume of 100 mL.
7. Store at 4 °C or freeze in aliquots.

PBS/Glycine 0.15% (w/v)

**Materials and equipment**

1. Balance
2. Erlenmeyer, 100 mL

**Reagents**

1. Glycine
2. H2O
3. PBS

**Procedure**

1. Pour 10 mL of 10x PBS in a 100 mL Erlenmeyer flask.
2. Add 0.15 g of glycine. Swirl until the glycine is dissolved.
3. Add H2O to a final volume of 100 mL.

Sucrose 2.3 M in PB (100mL)

**Materials and equipment**

1. Beaker 100 mL
2. Magnetic stirrer
3. Stirring bar
4. Vials 1 mL

**Reagents**

1. 0.1 M phosphate buffer
2. Sucrose D (+) Saccharose

**Procedure**

1. Mark the level for 100 mL in a beaker and add a stirring bar.
2. Add 78.73 g of sucrose.
3. Add 0.1 M phosphate buffer up to the marked level.
4. Stir until the sucrose is dissolved.
5. Aliquot into 1 mL vials.
6. Store at 4 °C.

Uranyl acetate 4% pH 4 (10 mL)

**Materials and equipment**

1. Erlenmeyer, 10 mL
2. Stirring bar
3. Magnetic stirrer
4. Millipore 0.45 µm filter
5. syringe

**Reagents**

1. H20
2. Uranyl acetate

**Procedure**

1. Add 0.4 g of uranyl acetate in 10 mL of dH2O.
2. Stir at RT in the dark till dissolved.
3. Store at 4 °C in the dark.

Uranyl oxalate acetate, pH 7 (100 mL)

**Materials and equipment**

1. Erlenmeyer flasks, 100 mL (3)
2. Magnetic stirrer
3. Millipore 0.45 µm filter
4. pH indicator sticks
5. Stirring bars
6. Syringe

**Reagents**

1. Ammonium hydroxide 25% NH4OH
2. H2O
3. Oxalic acid (2 H2O)
4. Uranyl acetate

**Procedure**

1. Prepare 4% uranyl acetate.
	1. Add 4 g of uranyl acetate in 90 mL of distilled water. Stir at RT in the dark until dissolved (2 h).
	2. Remove the stirring bar. Add distilled water to a final volume of 100 mL.
2. Prepare 0.3 M Oxalic acid.
* 2.1. Add 3.8 g of oxalic acid to 90 mL of distilled water. Stir at room temperature until dissolved (2 h).
	1. Remove the stirring bar. Add distilled water to a final volume of 100 mL.
1. Preparation of 2% UA in 0.15 M oxalic acid
	1. Add 50 mL of 4% uranyl acetate to 50 mL of 0.3 M oxalic acid and stir.
	2. Add NH4OH drop by drop till pH 7 is reached while constantly stirring. Check with a pH indicator stick.

NOTE: If too much NH4OH is added, the pH exceeds 8 and solid precipitates. Do not use this UOA!!! If the NH4OH is too old (too much CO3- in the bottle) the uranyl oxalate acetate will be neutral and will give less contrast and possible precipitates.

* 1. Filter through a 0.45 µm Millipore filter. Store at 4 °C in the dark.

Methyl cellulose-uranyl acetate, pH 4

**Materials and equipment**

1. Millipore 0.45 µm filter
2. Syringe 1 mL
3. Tube 10 mL with screw cap

**Reagents**

1. 2% Methyl cellulose in water
2. 4% Uranyl acetate in water

**Procedure**

1. Add 2 mL of the uranyl acetate solution (0.45 µm-filtered) to 18 mL of the methyl cellulose.
2. Mix gently.
3. Store at 4 °C in the dark (shelf life: 3 months).

Methyl cellulose 2%

**Materials and equipment**

1. Centrifuge
2. Erlenmeyer 250 mL
3. Graduated cylinder 200 mL
4. Magnetic stirrer
5. Stirring bar
6. Tubes (10 mL) with a screw cap

**Reagents**

1. H2O
2. Methyl cellulose 25 centipoises: [Sigma M-6385]

**Procedure**

1. Heat 196 mL of distilled water to a temperature of 90 °C.
2. Add 4 g of methyl cellulose while stirring. Rapidly cool the solution on ice while stirring.
3. Seal with Parafilm and stir O/N at 4 °C. Stop stirring, let the solution “ripen” for 2 days at 4 °C.
4. Add distilled water to a final volume of 200 mL.
5. Centrifuge for 1.5 h at 100,000 × *g*.
6. Pour the supernatant into tubes with screw caps.
7. Store at 4 °C in the dark (shelf life: 6–9 months).

Gelatin 12% for cell support (100 mL)

**Materials and equipment**

1. Erlenmeyer, 100 mL
2. Magnetic stirrer
3. Stirring bar
4. Vials 75 mL

**Reagents**

1. Gelatin powder, food quality
2. Na-Azide 20% (2 g/10 mL distilled water)
3. Phosphate buffer 0.1 M

**Procedure**

1. Add 12 g of gelatin to 75 mL of 0.1 M Phosphate buffer. Stir for 10 min at RT.
2. Warm the solution at 60 °C for 4–6 h (stir gently). When the gelatin has dissolved, cool the solution to 37 °C.
3. Add 100 µL of 20% azide.
4. Add 0.1 M Phosphate buffer to a final volume of 100 mL; stir gently.
5. Store in 7 mL vials at 4 °C.

BSA 10%

**Materials and equipment**

1. Centrifuge
2. Erlenmeyer, 100 mL
3. Graduated cylinders
4. Magnetic stirrer
5. pH meter
6. Stirring bar

**Reagents**

1. Bovine Serum Albumin Fraction V, [Sigma A-9647]
2. H2O
3. Na-Azide 20% (2 g in 10 mL of H2O), [Merck 822335]

**Procedure**

* + - 1. Add 10 g of BSA in 60 mL of dH2O (Milli Q) while stirring slowly. Stir slowly (to prevent foaming) O/N at 4 °C.
			2. Adjust the pH to 7.4 to with 1 M NaOH.
			3. Add 100 µL of 20% azide.
			4. Add dH2O to a final volume of 100 mL; stir gently.
			5. Centrifuge for 1 h at 100,000 × *g*.
			6. Store the supernatant in small aliquots at 4 °C. Dilute aliquots 10x for use.

1% BSA – C + 5% fish skin gelatin

**Materials and Reagents**

1. BSA-C 10% Aurion
2. Fish skin gelatin
3. 50 mL tube
4. PBS

**Procedure**

1. Heat fish skin gelatin to 37 °C.
2. Add 5 mL of BSA-C 10% to a 50 mL tube.
3. Add 5 mL of fish skin gelatin to the tube.
4. Ad 40 mL of PBS 1x and dissolve thoroughly.
5. Make aliquots. Dilute aliquots 10x for use.

Pickup solution

**Reagents**

1. Sucrose 2.3 M
2. Methylcelulose 2%

**Procedure**

* + - 1. Mix 1:1 methyl cellulose and sucrose at 4 °C.

1% GA in 0.1 M PB

**Reagents**

1. Gluteraldehyde (GA) 16% (Serva, 23115.01)
2. 0.2 M PB
3. H2O

**Procedure**

* + - 1. Add 7 mL of H2O to 8 mL of 0.2 M PB in a glass vial.
1. Add 1 mL of GA 16%.
2. Store at 4 °C.

Toluidine blue solution

**Reagents**

1. Toluidine blue (Merck, 1275)
2. Disodium tetraborate decahydrate
3. dH2O

**Procedure**

* + - 1. Dissolve 1 g of Toluidine blue and 1 g of disodium tetraborate decahydrate in 100 mL of dH2O.
			2. Pass through 0.45 µm filter directly before use.