# C42 Isolation of renal proximal tubule cells

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## 1. Background

Three types of renal cell have been purified using Nycodenz® gradients; the most commonly purified cell type is the proximal tubule cell, but cells from the thin limb of Henle and interstitial cells have also been isolated in these gradients.

Proximal tubule cells are used as an *in vitro* model for studies on nephrotoxicity and the method developed by Boogaard et al [1,2] yields 90-95% of proximal tubule cells with a high viability (97%). The perfusion technique is an important part of the methodology and is provided in the protocol, but for information about the preliminary ligation of blood vessels, the operator should refer to ref 1. As with all isolations of cells from tissues, the release of viable cells as a single cell suspension by disaggregation of the tissue with collagenase is a critical part of the procedure.

Interstitial cells and thin limb of Henle cells from the disaggregated inner medulla have been purified in two steps. Firstly, they are separated from inner medullary collecting duct cells using magnetic beads coated with Dolichos Biflorus Agglutinin, which binds almost exclusively to the collecting duct cells. Subsequently purification of the non-collecting duct cells is achieved in a continuous Nycodenz® gradient with a density range of 1.052-1.093 g/ml; this is approximately equivalent to 9.4%-17% Nycodenz® [3-5]. After centrifugation at 1500g for 45 min the interstitial cells band at 1.081-1.093 g/ml, approx. equivalent to 15-17% Nycodenz® [3,4]. In this same gradient, the thin limb of Henle cells band predominantly at 1.052-1.069 g/ml, approx equivalent to 9.4%-12.5% Nycodenz® [5].

The following protocol for isolation of proximal tubule cells is adapted from ref 1 (see Notes 1 and 2).

## 2. Solutions required

**A.** Perfusion medium 1: Calcium-free Hank's Balanced Salt Solution containing 0.5 mM EGTA and 25 mM HEPES

**B.** Perfusion Medium 2: As solution A without EGTA.

**C.** Perfusion Medium 3: As Solution B containing 4 mM $CaCl_2$ and 0.12% collagenase

**D.** Perfusion Medium 4: As Solution B containing 2.5% (w/v) bovine serum albumin (BSA)

**E.** Nycodenz® stock: 60% (w/v) in water (see Note 3)

**F.** Nycodenz® diluent: 67 mM KCl, 12.2 mM $CaCl_2$, 100 mM HEPES-NaOH, pH 7.4

**G.** Nycodenz® (34%, w/v) solution: Mix 3.4 vol. of Solution E with 0.6 vol. of Solution F and 2 vol. of water

**H.** Low density barrier solution: mix 1 vol. of solution G with 4 vol. of Solution D (see Note 4).

Keep the following stock solutions at 4°C
1 M HEPES (free acid): 23.8 g per 100 ml water; adjust to pH 7.4 with 1 M NaOH before making up to final volume.
100 mM EGTA: 3.80 g per 100 ml water (pH 11-12)
1 M KCl: 7.46 g per 100 ml water
1 M $CaCl_2•2H_2O$: 14.7 g per 100 ml water

Prepare Solutions A-D from commercially available 10x calcium-free Hank's Balanced Salt Solution. Dilute this with approx half of the required water before adding the HEPES stock solution plus the other supplements as appropriate. Then make up to the full volume with water. Gas the solutions with 5% $CO_2$/air, the pH should be adjusted to 7.4.

Solution F: To 50 ml of water add 10 ml, 6.7 ml and 1.22 ml respectively of the HEPES, KCl and $CaCl_2$ stock solutions; adjust to pH 7.4 and make up to 100 ml with water

## 3. Protocol

Carry out Steps 1-3 at 37°C and step 5 onwards at 0-4°C. Keep Solutions D, G and H at 0-4°C.

1. Perfuse the kidneys with 150 ml of Solution A at 37°C at 10 ml/min; once all the blood has been washed out reduce the flow rate to 7.5 ml/min.

2. After removing kidneys, continue perfusion with 25 ml of Solution B.

3. Perfuse in a recirculating system with Solution C for 18 min.

4. Wash out Solution C with 10 ml Solution D.

5. Remove the capsule and disperse the tissue in Solution D.

6. Filter the cell suspension through two layers of nylon gauze (80 mesh).

7. Centrifuge the cells at 80*g* for 3 min and wash the pellet three times in Solution D (see Note 5).

8. Resuspend the cells in 4 ml of Solution D and mix with 2 ml of Solution G.

9. Overlayer 3 ml of the cell suspension with 1 ml of Solution H and 0.5 ml of Solution D (see Note 6).

10. Centrifuge at 2300*g* for 3 min (see Note 7).

11. Harvest the proximal tubule cells from the lower interface.

## 4. Notes

1. A number of other published papers have also reported the use of this methodology for the isolation of rat renal proximal tubule cells [6-11], more or less as described by Boogaard et al [1,2]. There have however, been some small modifications to both the buffers and the centrifugation conditions. Schaff et al [12] used a Hank's Balanced Salt Solution (HBSS) containing PIPES rather than HEPES; these workers also suspended the cells in 14% (w/v) Nycodenz® rather than 11.3% Nycodenz® and 9% Nycodenz® was layered on top rather than 6.8% Nycodenz®.
2. Kruidering et al [13] obtained the cells from pigs and used a perfusion medium (Eurocollins, pH 7.4) comprising 177 mM glucose, 10 mM $NaHCO_3$, 15 mM KCl, 42 mM $K_2HPO_4$, 15 mM $KH_2PO_4$ and 2 mM glycine. In this case, the minced cortex was washed with $Ca^{2+}/Mg^{2+}$-free HBSS containing 25 mM HEPES and 2 mM glycine. Disaggregation of the minced tissue was achieved in 0.07% collagenase in the same HBSS solution supplemented with 4 mM $CaCl_2$ and 1 mM deferoxamine. The density gradient was rather different, being composed of 8.5% (5 ml), 11.3% (10 ml) and 17% (w/v) Nycodenz® (5 ml); the cells being in the 11.3% Nycodenz® layer. The centrifugation time was 6 min and the proximal tubule cells were recovered from the upper interface.
3. Keeping Nycodenz® as a sterile stock solution of 60% (w/v) in water is a convenient source for preparation of a broad range of gradients. Make the stock solution by adding the powder slowly to water at approx 60°C while stirring, then allow the solution to cool to room temperature before making up to the desired volume. Its refractive index should be 1.4318 and its density 1.31 g/ml. Sterilize the solution by filtration.
4. This 6.8% Nycodenz® solution has a density of approx 1.037 g/ml.
5. Carry out the resuspension of the cells after each centrifugation very gently, with the minimum of shearing forces.
6. For more information about making gradients see Basic Techniques File 2a. ➔➔
7. Do not use the brake to decelerate the rotor.

## 5. References

To access abstracts of refs 1-13 (file CA42) click on the double blue arrow ➔➔


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