Primary cell cultures from pituitary of Dicentrarchus labrax

1. Specimens of European sea bass *Dicentrarchus labrax* are anesthetized with 2-phenoxyethanol diluted 1:10000 in seawater and then sacrificed by decapitation.
2. Collect pituitaries using sterile tweezers and place them in HBSS supplemented with antibiotics. Clean them from adherent tissues using a scalpel and tweezers.
3. Mince tissues manually to small fragments using sterile razor blades.
4. Fragments are digested with 1% collagenase for 30 min at room temperature under agitation. Let the fragments settle and collect the first supernatant.
5. Repeat step 4 and collect the second supernatant. Pool both supernatants in 3 ml of HBSS supplemented with 1X penicillin/streptomycin.
6. Centrifuge 5 min at 1000g. Remove the digestion solution and wash twice with HBSS supplemented with 1X antibiotics.
7. Resuspend the pellet in 2 ml of cell culture medium L15 supplemented with 10% FBS and 1X antibiotics. Count cells.
8. Place cells in the cell culture plates and incubate at 25°C.

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Dicentrarchus labrax: adult specimens bred in aquaculture conditions.

Cell culture apparatus: cooled cell incubator and a class II biological safety cabinet.

Cell culture medium: Leibovitz's medium (L15) supplemented with 10% fetal bovine serum FBS (GIBCO 10270) and 1X antibiotics: penicillin 100 units, streptomycin 0.1 mg/ml.

Solutions: Hank's balanced salt solution (HBSS): KCl (0.40 g/l), KH_2PO_4 (0.06 g/l), NaCl (8 g/l), $NaHCO_3$ (0.35 g/l), Na_2HPO_4 (0.05 g/l), D-glucose (0.91 g/l), HEPES (3.60 g/l), pH 7.2.

Digestion solution: 1% type I collagenase, penicillin (100 units), 0.1 mg/ml streptomycin (1X) in HBSS.

Plasticware: 12-well and 6-well plates, polyesther membrane inserts.

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All chemicals were purchased from Sigma-Aldrich unless otherwise stated.

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