

Primary chorion and decidua cells isolated from fetal membranes

From: Duke/UNC/UT/EBI ENCODE group

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Source: Dr. Amy Murtha at Duke University (Durham, NC).

Lineage: Fetal membranes were collected from women who underwent planned cesarean delivery at term, before labor and without rupture of membranes. Institutional review board approval was obtained for waiver of consent to obtain deidentified tissue that was not to be used for clinical purposes. Tissue was transported to the laboratory in Dulbecco's Modified Eagle Medium-Hams F12 (DMEM/F12) media.

Karyotype: unknown

Isolation of chorion and decidua cells: Fetal membrane tissue was cut into 2 × 2-inch squares with forceps and scalpel. The smooth layer of amnion was pulled off manually. Separation of the decidua and chorion involved blunt dissection with forceps and scalpel. Each layer was minced by cross cutting with scalpel blades. Tissues were processed in digestion buffer I at 37°C for 30 minutes, followed by centrifugation at 2000 rpm for 10 minutes. The cell pellet was resuspended in digestion buffer II and incubated for 60 minutes at 37°C. Cells were filtered through four layers of sterile gauze and centrifuged at 2000 rpm for 10 minutes. A cell separation gradient was prepared by using an Optiprep column (Sigma Aldrich) with steps ranging from 4% to 40%, of 4 mL each (4%, 6%, 8%, 10%, 20%, 30%, 40%). Processed chorion or decidua cells were added to the top of the gradient, then centrifuged (1000×g) at room temperature for 30 minutes. Cells between densities of 1.049 and 1.062 g/mL represented the chorion layer. Cells between densities of 1.027 to 1.038 g/mL represented the decidual layer. Harvested cells were washed with DMEM/F-12, centrifuged and resuspended in DMEM/F-12. Cells are not plated or passaged.

Digestion buffer I

0.125% trypsin (Gibco Cat # 15050-065) in Hank's Balanced Salt Solution (pre-prepared from Gibco/Invitrogen)

0.02% DNase I (Sigma Cat No: D4527)

Digestion buffer II

0.125% trypsin (Gibco Cat # 15050-065) in Hank's Balanced Salt Solution (pre-prepared from Gibco/Invitrogen)

0.02% DNase I (Sigma Cat No: D4527)

0.2% collagenase, type IA (Sigma Aldrich, St. Louis, MO, Cat Number C2674)

Resuspension media

DMEM/F-12 (Lonza Cat No: 12-719F)

FBS: Sigma F2442