Differentiation Protocol

Cat. T0863

Immortalized Bovine Chromaffin Cells (BADA.20)

Culture conditions recommended for the differentiation of Bovine Chromaffin Cells (BADA.20):

Differentiation Media (DM): DMEM/F12 (Gibco) + 1% Bovine Serum Albumin (Sigma) + 1X TCM serum replacement (VWR) + 1% Penicillin/Streptomycin (G255) + 5 µg/ml Dexamethasone (Sigma) + 500 µM tetrahydrobiopterin (Sigma). Incubate at **37 degrees Celsius, 5% CO_2**.

Culture conditions recommended for the proliferation of Bovine Chromaffin Cells (BADA.20):

Proliferation Media (PM): DMEM/F12 (Gibco) + 10% Fetal Bovine Serum (Sigma) + 125 µg/ml Gentamicin (Sigma) + 1% Penicillin/Streptomycin (G255). Incubate at **33 degrees Celsius, 5% CO_2.**

Cells need to be purified by differential plating to remove fibroblasts before freezing/storage or differentiation for further use (e.g. immunostaining or transplantation). See below for the differential plating protocol.

Differential Plating Protocol:

Day 1:

1. Remove cells from culture flask with 0.5 mM EDTA.
2. Replate cells in 10ml PM (Proliferation Media) into “non tissue culture treated” plates (100 mm petri dish)
3. Incubate 4h at 33°C
4. Remove supernatant into 25 cm2 flask
5. Incubate 2h at 33°C
6. Remove supernatant into 25 cm2 flask
7. Incubate overnight at 33°C

Day2:

1. Remove supernatant into 15 ml tube
2. Wash flask with 0.5-1ml of 0.5 mM EDTA in DPBS to remove attached cells
3. Add 3 ml of PM to wash the flask and transfer into 15 ml tube

4. Spin 5 min @500g
5. Replate into “non TC treated” plate for 4h at 33°C
6. Repeat steps 4-7 of Day 1, adjust the volume of media needed

To differentiate cells:

Remove supernatant and detach adherent cells with 0.5 mM EDTA in DPBS. Resuspend pellet in differentiation media and plate into flasks (25-75 cm^2) Culture for up to 10 days at 37°C, change media every 3-4 days. Cells can then be verified for differentiated chromaffin cell phenotypes using immunostaining or other desired methods.

This protocol has been adapted from the publication: Eaton et al, 2000, Generation and Initial Characterization of Conditionally Immortalized Chromaffin Cells, Journal of Cellular Biochemistry 79:38–57 (2000).

Updated on March 27, 2023