HC11 Culture

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Summary

HC11 was isolated as a prolactin-responsive cell clone, HC11, from the COMMA-1D mouse mammary epithelial cell line (Ball et al., 1988). Clone HC11 was selected as a unique example of a cloned mouse mammary epithelial cell which has no requirement for complex, exogenously added, extracellular matrix or co-cultivation with other cell types for the prolactin-dependent in vitro induction of the endogenous beta-casein gene by lactogenic hormones.

Culture Medium preparation

1. In 1 liter beaker with a stir bar, add

800 ml milliQ water	
RPMI 1640 (31800105, Invitrogen)	1 bag
HEPES powder (BP310-500, Fisher)	5.95 g (25 mM final)
NaHCO3 powder (S233-500, Fisher)	2.0 g
Pen-Strep aliquot (100x) (15140-122, Invitrogen)	10 mL

2. Adjust pH to 7.1-7.2 with 0.5M NaOH while stirring

3. Bring volume to 900 ml

4. Add the following:

Insulin (5 mg/ml in PBS) (I6634-250MG, Sigma)	1 ml	(5 µg/mL final)
EGF (100 µg/ml) (01-107, Millipore)	0.1 mL	(10 ng/mL final)
Heat-inactivated FBS (varies)	100 ml	(10% final)

5. Mix well again by stirring

6. Filtered through 500 ml cup filter (0.22 µm, SCGP-T05-RE, Millipore) to TC bottles (200 ml/bottle)

7. Working media at 4 C; freeze unused media – can be stored in -20 ºC for months before use

Routine culture (for 100-mm dish)

Feed with fresh medium every 2-3 days and passage before the plate gets over confluent. Usually split the cells every 3-5 days at 1:10 dilution.

1. wash twice with Ca^{2+}-free PBS (5-10 mL).

2, add 2 mL of 0.25% Trypsin/EDTA and incubate for 5-10 min at 37ºC.

3, Neutralize Trypsin by adding 5-mL of culture medium, then mix well, and centrifuge at 1000 rpm for 5 min.

4, aspirate supernatant and split the cells with 8-10 mL of fresh medium/dish.

Frozen stocks

Resuspend 1-2 million cells in 1ml culture media with 10% DMSO, store in sealed Styrofoam at -80ºC for at least overnight, then transfer to plastic box in liquid nitrogen tank.

Induction of differentiation

1. HC11 cells are distributed in 6-well plates at $5x10^5$/well and allowed to reach confluency.
2. Change medium to HC11 medium without EGF for 48 hours to induce competence
3. Differentiation is induced with HC11 medium without EGF but containing fresh added 5 µg/ml insulin (same as above), 100 nM dexamethasone (D4902-500MG, Sigma), and 5 µg/ml prolactin (L6520-250IU, Sigma) for 3 days.
4. Differentiation is monitored by quantification of beta-casein expression with Q-PCR over time.

- for 5mg/ml prolactin stock (1000x), dissolve 3 mg prolactin in 150 µl 0.001 N NaOH, then add 450 µl HC11 medium without EGF, store in -20 ºC.
- for 1mM Dexamethasone (10Kx), dissolve 3.9 mg in 10 ml ethanol, aliquot and store in -20 ºC

References

Ball, R.K., Friis, R.R., Schoenenberger, C.A., Doppler, W., and Groner, B. (1988). Prolactin regulation of beta-casein gene expression and of a cytosolic 120-kd protein in a cloned mouse mammary epithelial cell line. The EMBO journal *7*, 2089-2095.