Culture of BEND Cells (Bovine Endometrial Cells)

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This protocol was adapted from one prepared by Aydin Guzeloglu and William Thatcher. The cell line was prepared by T.R. Hansen, University of Wyoming and is available from ATCC.

Culture Medium

Ingredients

these catalog numbers refer to products from Sigma; products from other suppliers can also be used

Powdered Hams F-12 (N6760)
Powdered MEM D-valine modification (M7395)
Fetal bovine serum (F4135)
Horse serum (H1138)
Antibiotic-antimycotic (ABAM) (A9909)
D-valine (V1255)
Insulin (I5500)

Preparation

Place 1000 ml of double distilled water in 4000 ml beaker.
2. Add 10.7 g of F-12 and 9.62 g of MEM, allow to dissolve
3. Add 400 IU of insulin
4. Dissolve 0.0683g of D-valine in 1 ml of 0.5 N NaOH
5. Add the 1 ml valine to culture mix
6. Add 3.37 g of sodium bicarbonate
7. Adjust pH to 7.3
8. Bring total volume up to 1600 ml with double distilled water
9. Filter 4 aliquots of 400 ml into 500 ml bottles
10. Store medium at 4 C until needed
11. To make medium containing serum (for culture), add 50 ml of FBS, 50 ml of horse serum and 5 ml of ABAM to each 400 ml bottle

Complete medium can be stored at 4 ^{o}C for 2 weeks.
Medium must be warmed for a minimum of two hours prior to use.

Thawing Cells

1. Remove ampule from LN_2 storage tank and place in 37^{o} C water bath
2. Invert sample every 30 seconds and place back into water bath until sample is thawed.
3. Pour contents of storage vial into T175 culture flask.
4. Add 50 ml of complete medium and place in 37^{o} C incubator for 24 hours.
5. Remove old medium and replace with 50 ml of fresh medium.
6. Place back in incubator until cells become confluent (normally 2-4 days).
7. Once cells reach >90 confluency, they are ready to be trypsinized.

Trypsinization/Splitting

1. Remove old medium
2. For T175 flasks add 20 ml of trypsin; for T75 flasks add 10 ml of trypsin.
3. Place back into 37 ^{o} C for 10 min and tap flask gently to dislodge cells.
4. If cells are not completely trypsined, add another 10 ml of trypsin and repeat step 3.
5. Once cells are dislodged, add 10 ml of complete medium to stop trypsinization.
6. Pour contents into 50 ml centrifuge tube and centrifuge for 10 min at 1000 RPM.

7. Remove old medium, being careful not to dislodge pellet.
8. Resuspend pellet in complete/serum medium: 4 ml for T175 flask and 2 ml for T75 flask
9. Transfer 1 ml cells/medium to T75 flask or 2ml cells/medium to T175 flask.
10. Add 30 ml complete/serum medium to T75 flask or 50 ml to T175 flask.
11. Place in 37 ^{o}C until confluency
12. Repeat trypsinaztion/splitting procedure

Freezing

1. Perform trypinization procedure to step 8.
2. Using hemacytometer, calculate cell number.
3. Dilute cells to concentration of 2 million cells per ml of medium.
4. Slowly drip in equal volume of 20% (v/v) DMSO solution in culture medium
5. Allocate cells in 1 ml cryotubes. On each tube make note of date name and passage number.
6. Place at -70 ^{o}C for 24 hours.
7. Store cells in LN_2.
8. Record storage location, passage number, date, and name of person freezing cells in the cell bank log.
9. Thaw and test 1 sample per freezing run to test viability.

References using BEND cells

Johnson et al. Endocrine 10:243 (1999)
Perry et al. Mol. Endocrinol. 13: 1197 (1999)
Austin et al. Endocrinology 140: 542 (1999)