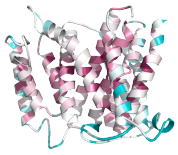
Cell Culture

Media Requirements



Chinese Lung Fibroblasts:

- **CCL39, PS120** – DMEM High Glucose with Ab & Am
- **PSN, KR/A and E266I** (NHE add-back): DMEM High Glucose with Ab & Am AND G418 (0.4 mg/ml)
- **PS120 with NHE-DDK, Rsk, Rock, Rock/Risk (NHE add back)** DMEM with G418.

Human Non Small Cell Lung Cancer:

- **H358, H460, H1299, H69AR** – RPMI with Ab but NO Am
- **A549** – DMEM High Glucose with Ab but NO Am
- **1299 and 460 NHE KD or CHP2 KD and shUPAR KD** (RPMI puromycin)

Human Lung Fibroblast Cells:

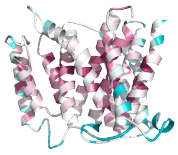
- **Ha888Lu** DMEM without any antibiotics or antimicotics.

Human Breast Cancer Cells:

- **MCF7 –** RPMI with Ab but NO Am
- **MDA-MB-231**– RPMI with Glutamine without Ab or Am

Human Prostate Cancer Cells:

- **RWPE (Normal Prostate Cells)** – Keratin free KSF-< from INvitrogen
- **LNCaP (CRL1749), Du145 (HTB81), & PC-3 (CRL1435)** - DMEM High Glucose w/ Non Essential Amino Acids & Ab but NO AM



Antibiotics - Quick info and Mechanism.

Stable Transfection: *Some (very few) of the transfected cells will, by chance, have integrated the foreign genetic material into their genome. If the toxin is then added to the cell culture, only those few cells with the marker gene integrated into their genomes will be able to proliferate, while other cells will die. After applying this selective stress (selection pressure) for some time, only the cells with a stable transfection remain and can be cultivated further using a lower concentration (maintenance). THUS the antiobiotic cultured with the media depends on the gene transfected into the cells.*

G418 – AKA Geneticin (similar but NOT the same as gentamicin), blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the neo gene from Tn5 encoding an aminoglycoside 3'-phosphotransferase. Selection in mammalian cells is usually achieved in three to seven days with concentrations ranging from 400 to 1000 µg/ml. Cells that are dividing are affected sooner than those that are not. Molecular weight: 692.7

Puromycin specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes. Resistance to puromycin is conferred by the Pac gene encoding a puromycin N-acetyl-transferase (PAC) that was found in a *Streptomyces* producer strain. Animal cells are generally sensitive to concentrations from 1 to 10 µg/ml. Molecular weight: 471.51

Penicillin/Streptomycin (Pen/Strep) – Not used for selection but to inhibit bacterial growth- standard antibiotic mixture used against a wide variety of Gram-positive and Gram-negative bacterial organisms. It has no activity against fungi or yeast. Typically purchased as 100 x concentrated stock. ALWAYS double check before adding to media. . Use for CCLI cells, NOT FOR HUMAN CELLS.

Amphotericin B (antimicotic) Interferes with fungal membrane permeability by forming channels in the membranes of yeast and fungus, causing small molecules to leak out. Antimicrobial spectrum: Yeasts and molds. Caution: Stable at 37 °C for 3 days. 5.6 mg (solid)/L

Doxycycline (Dox)- Tet System Inducers is a derivative of tetracycline, is the recommended inducer for Tet Systems. Dox can be used at 100-fold lower concentrations and has a longer half-life than tetracycline.

NHE-RFP containing cells carry the Puromycin resistant genes.
NHE-PSN (DDK) and it's mutant variants (Rsk/Rock/Rsk&Rock) all carry G418 resistance.
SH RNA knockdowns are in Puromycin resistant vectors ***CHP NHE shUPAR KD***

DMEM Selection media (10% FBS) working concentration = 1mg/ml G418

DMEM Maintenance media (10% FBS) working concentration = 0.250 mg/ml G418

Stock concentration 50mg/ml - Located in 4°C in culture room

RPMI Selection media (10% FBS)- working concentration = 3ug/ml Puromycin

RPMI Maintenance media (10% FBS)- working concentration =1ug/ml Puromycin

Stock concentration 10 mg/ml - prepare sterile in water. Store at -20°C

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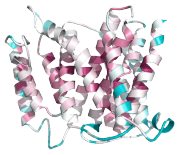


NCI-H460 (HTB-177) Human carcinoma; large cell lung cancer. Derived from the pleural fluid of a patient with large cell cancer of the lung. The cells express easily detectable p53 mRNA at levels comparable to normal lung tissue, and exhibit no gross structural DNA abnormalities. The cells stain positively for keratin and vimentin but are negative for neurofilament triplet protein.

- Add Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
- Note: ***To avoid clumping do not agitate*** the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

NCI-H1299 (CRL-5803) Human carcinoma; non-small cell lung cancer. Derived from metastatic site: lymph node. The cells have a homozygous partial deletion of the p53 protein, and lack expression of p53 protein.

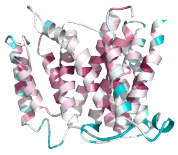
- Add Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
- Note: ***To avoid clumping do not agitate*** the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

NCI-H358 (CRL-5807) Human lung bronchioalveolar carcinoma; non-small cell lung cancer. Derived from tumor tissue obtained from a patient prior to initiation of chemotherapy. Ultrastructural studies demonstrated the presence of cytoplasmic structures characteristic of Clara cells. They have a reported colony forming efficiency of 0.83% in soft agarose.

Hs 888.Lu (CRL-7624) The line was established from apparently normal tissue from a patient who had metastatic osteosarcoma. **Subcultivation Ratio:** A subcultivation ratio of 1:2 is recommended.

CCL-39 Cricetulus griseus (hamster, Chinese) lung fibroblast. Virology. 1964 Apr;22:439-45. VIRUSES AND MAMMALIAN CHROMOSOMES. I. LOCALIZATION OF CHROMOSOME ABERRATIONS AFTER INFECTION WITH HERPES SIMPLEX VIRUS.

A549 (CCL-185) Human Lung carcinoma This line was initiated through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. A549 cells are positive for keratin.



Media Labeling System

For 125 ml culture bottles

ALL labels will include the following information:

- DMEM or RMPI
- Pen/Strep and or AM (antimicotic) if appropriate
- % of FBS write 10% FBS or 0.5% FBS
- BASE = equals medium without serum. ***NO antibiotics added to the base will be indicated and NOT assumed.***
  Thus if you add Pen/Strep to the base, write it on the bottle.

DMEM Bottles	**RPMI Bottles**
10% FBS use yellow tape	10% FBS use red tape
0.5%FBS use blue tape	0.5%FBS use green tape

MEDIA WITH ANITBIOTIC – G418 or Puromycin: will have white tape AND the name of the antibotic with either Maint or Selection media. Use both white tape and the correct color tape to indicate the kind of media.