



MARC-145 Cell Culture

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2015.4.13



Cell Culture Basics

- ❖ Porcine alveolar macrophages (PAM) and MARC-145 cells are known to be permissive to PRRSV infection and replication.
- ❖ The MARC-145 is routinely used for in vitro propagation of PRRSV and for large scale production of PRRSV vaccine strains.



Cell Culture Basics

MARC 145 Cell line

Cell Lines	MARC 145
Organism	<i>Cercopithecus aethiops</i> African green monkey
Tissue	kidney
Cell Type	epithelial
Culture Properties	adherent
Virus Susceptibility	PRRSV

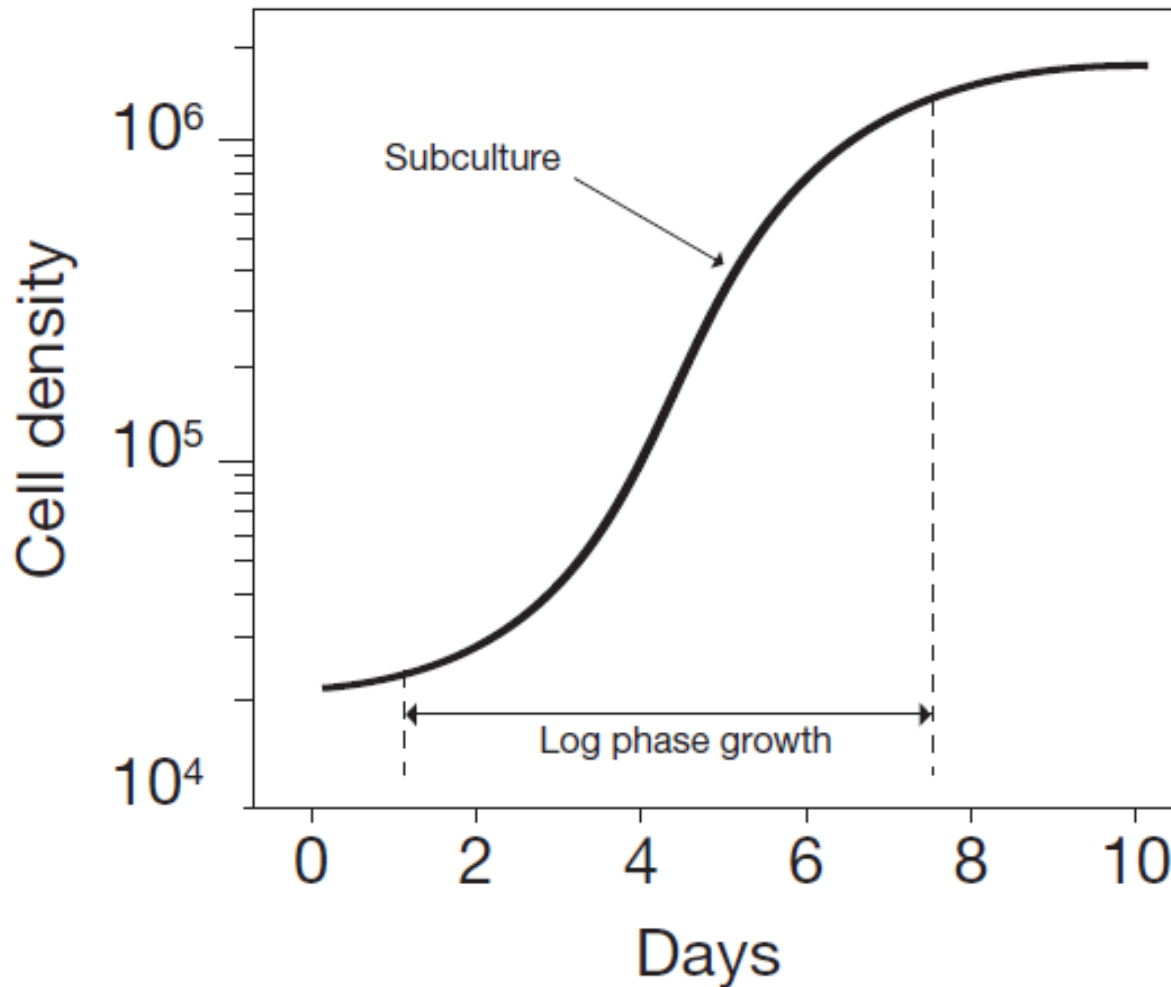


Cell Culture Basics

MARC 145 Cell Culture Environment

Cell Lines	MARC 145
Basal Media	DMEM (Dulbecco's Modified Eagle Medium)
Serum	5-10%FBS (Fetal bovine serum)
PH	7.4
CO ₂	5%
Temperature	37°C
Subculture Ratio	1:3-1:5
Medium Renewal	Every 2 to 3 days.

Cell Growth and Propagation





Cell Growth and Propagation

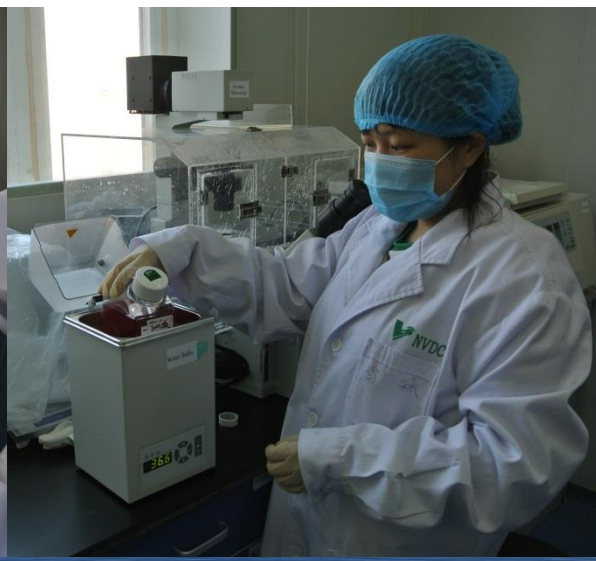
❖ Materials Needed

- Tissue-culture treated flasks, plates or dishes
- Complete growth medium
- Water bath 37°C
- Disposable, sterile pipettes
- Incubator with humid atmosphere of 37 °C 5% CO₂
- Inverted microscope
- 0.25% Trypsin-EDTA
- Automated cell counter

Cell Growth and Propagation

❖ Protocol

- Put on gown and gloves, turn on cabinet and swab all internal surfaces of the cabinet with 70% alcohol.
- Prepare Complete Growth Medium for the cell line. Medium should be at 37°C.
- Observe cell monolayer.



Cell Growth and Propagation

❖ Protocol

- **Remove** and discard the cell culture media.
- **Wash** the cell layer with 1ml trypsin-EDTA solution
- Add 1ml trypsin-EDTA solution to the flask
- **Incubate** the culture vessel at 37°C for 5-15 minutes



Cell Growth and Propagation



❖ Protocol

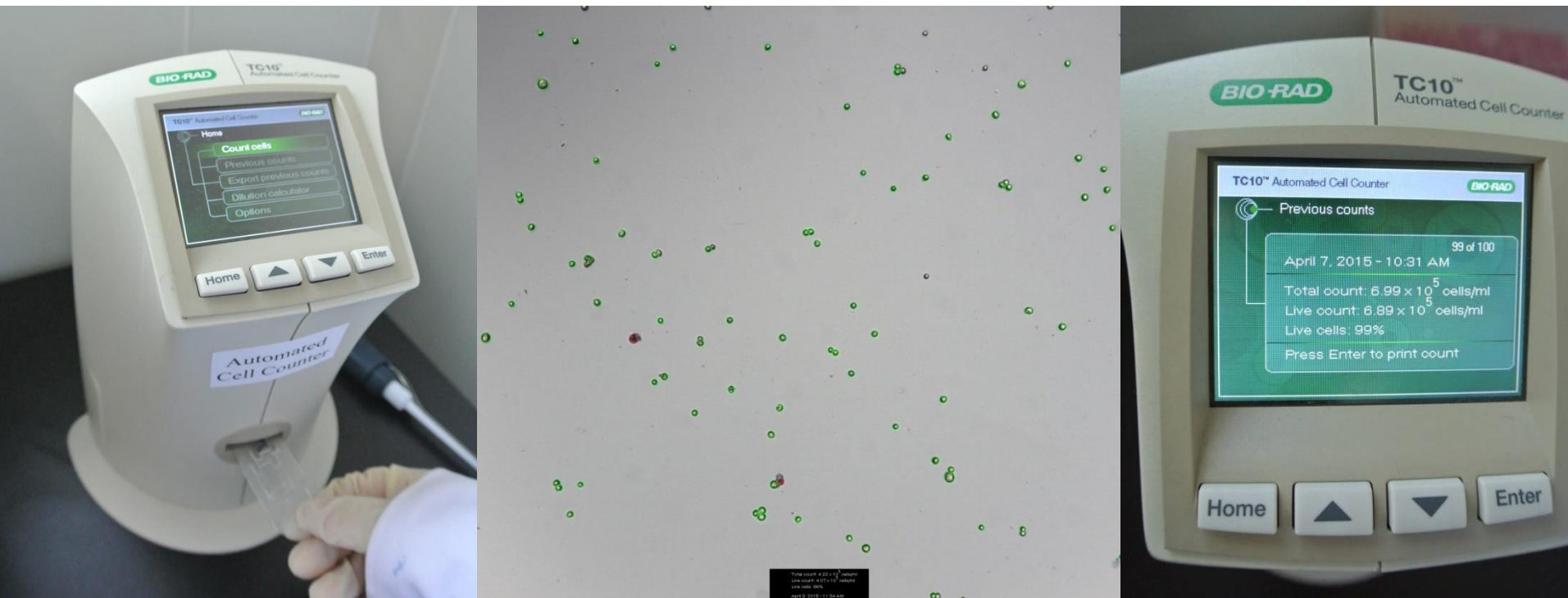
- **Observe** the cells by inverted microscope for detachment.
- **Inactivate** trypsin with 4ml complete growth medium



Cell Growth and Propagation

❖ Protocol

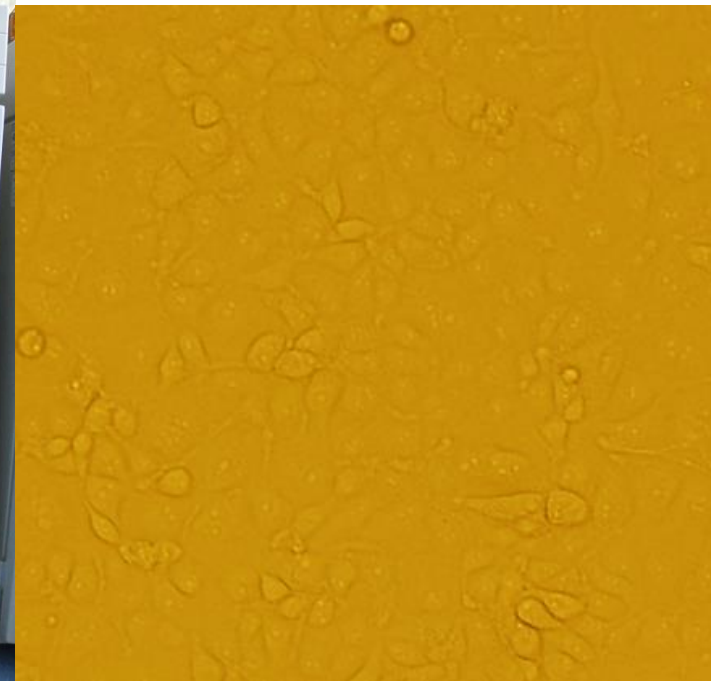
- Count the cells in suspension
- Inoculate cells to culture vessels



Cell Growth and Propagation

❖ Protocol

- **Incubate** the cells at 37°C 5% CO₂
- Every 2 to 3 days, subculture ratio is 1:3 to 1:5.





Cell Growth and Propagation

❖ Precautions

- Cells should be passaged when they are in the log phase
- Prewash cells is necessary.
- Prewarming of the enzyme solution will decrease the exposure period
- Trypsin need to inactivate with serum.
- To be sure that most ($>95\%$) are single cells.
- Always use proper aseptic technique and work in cell culture hood.
- Always wear personal protective equipment

Thank you !

