
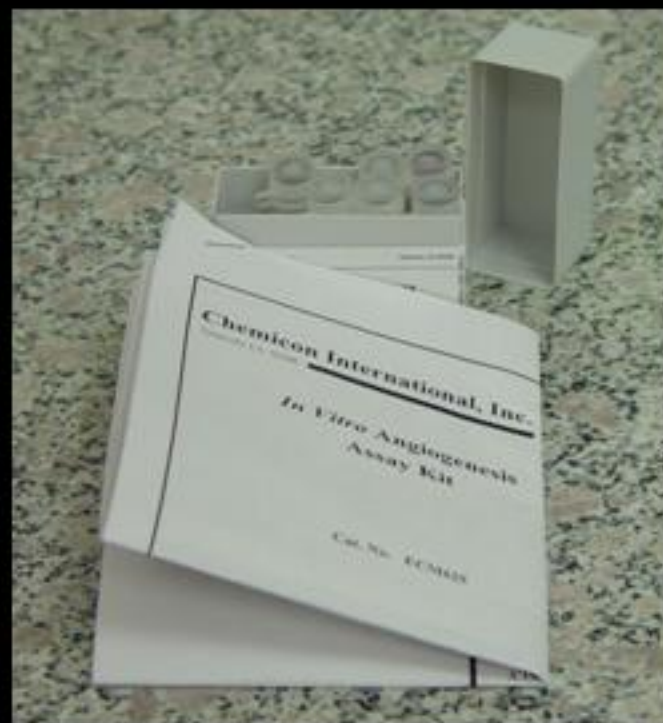


CHEMICON'S

**In Vitro  
Angiogenesis Assay  
(ECM625)**



## A rapid method to measure endothelial cell tube formation using researcher cell lines



### Kit Components:

- ECMatrix™ Gel Solution
- ECMatrix™ Diluent Buffer

# In Vitro Angiogenesis Assay

## Materials required but not provided:

- Endothelial cells HUVEC, etc ( $5 \times 10^3$ )
- 96-well Tissue Culture Plate
- Multichannel pipette and tips
- Microcentrifuge tubes, sterile
- 37°C CO<sub>2</sub> Incubator
- Inverted Light Microscope

## Advantages:

FLEXIBLE - 96-well design

CONVENIENT – Ready to use ECMatrix™

VERSATILE – Useful for multiple studies

INEXPENSIVE – Cost Effective Method of Experimentation



## Preparation of ECMatrix™

**Step 1: Thaw ECMatrix™ Solution and Diluent Buffer on ice or 0° water bath.**





Step 2: Add 100  $\mu\text{L}$  of 10X Diluent Buffer to 900  $\mu\text{L}$  of ECMatrix™ Solution in a sterile microfuge tube. Mix well.

**(Keep solution on ice to avoid ECMatrix™ solution polymerization.)**





Step 3. Transfer 50  $\mu$ l of diluted ECMatrix™ into each well of a 96-well tissue culture plate.





**Step 4. Incubate plate at 37°C for at least one hour to allow the matrix solution to solidify.**





**Step 5. Harvest experimental endothelial cells and resuspend in the appropriate culture media.**





Step 6. Seed  $5 \times 10^3$  -  $1 \times 10^4$  cells per well onto the surface of the polymerized ECMatrix™.





**Step 7. Incubate at 37°C in a CO<sub>2</sub> incubator. Cells cultured longer than 16 hours will begin to undergo apoptosis**



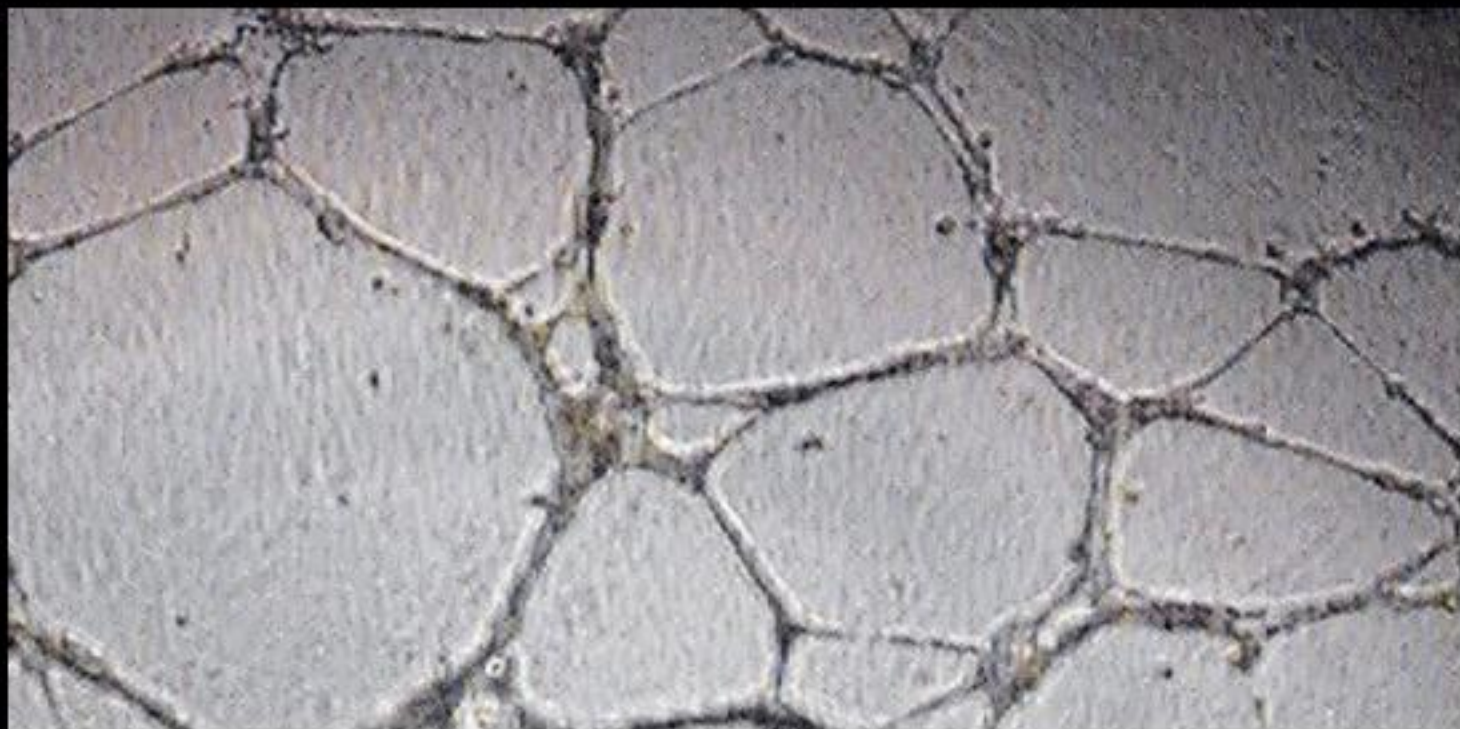


Step 8. Inspect tube formation under an inverted light microscope at 20X-100X magnification.





Endothelial cell tube formation will resemble figure below:



HUVEC cells incubated at 37°C on ECMatrix®