# MAPTrix HyGel™, the First Biochemically Defined 3D Extracellular Matrix

## MAPTrix™ Angio Kit for Tube Formation Assays

### Overview

Angiogenesis, the formation of new blood vessels, depends upon specific molecular interactions between endothelial cells and components of the extracellular matrix (ECM). Endothelial cell adhesion to the ECM is mediated by cell surface receptors, primarily integrins. The integrin-mediated adhesion leads to intracellular signaling events that regulate endothelial cell survival, proliferation, migration, and tube formation[1].

The MAPTrix™ Angio Kit provides a 3D (3-dimmensional) microenvironment to regulate the integrin-mediated signaling event for angiogenesis in a reliable and reproducible manner.

### Highlighted Features

The MAPTrix™ Angio Kit is designed as a cost effective and efficient method for tube formation assay. The Kit can be customized to design specific biochemical compositions for specialty applications.

- **Customized System:** Biochemically user-defined 3D matrix
- **Flexibility:** 24-, 48-, or 96-well design
- **Convenience:** Ready-to-Use Formula
- **Versatility:** Useful for multiple studies
- **Lot to Lot Consistency:** Reproducible & reliable results

### Product Description

MAPTrix™ Angio Kit is designed to *in situ* create anartificial basement membrane that mimics a native basilarlamina for tube formation assay.

The Basic Kit consists of MAPTrix™ ECM containing IKVAV, the key integrin in angiogenesis (αvβ3) binding peptide motif, and one or more peptide motifs that interact with angiogenic integrins such as α2β1 or α5β1. Presentation of at least two different angiogenic integrin-binding peptide motifs can generate an ECM microenvironment where combinatorial integrin mediated signaling can be induced for angiogenesis.

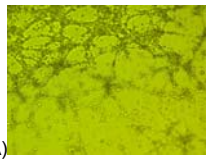
### Application

The tube formation is the mostly widely used *in vitro* assay model of reorganization stage of angiogenesis[2,3].

The MAPTrix™ Angio Kit can be used to monitor the extent of tube assembly in various endothelial cells, for example, human umbilical vein cells (HUVEC) or bovine capillary endothelial (BCE) cells.

The Kit can be also used as a high-content assay for evaluation of integrins as regulators of angiogenesis or screening anti-angiogenic drug targeting integrins.

**Figure1:** HUVEC tube formation on MAPTrix™ Angio

(A)  (B) 

Human umbilical vein endothelial cells (HUVEC) (40,000 viable cells/cm$^2$) were seeded on a 48-well polystyrene plate coated with a biochemically defined 3D matrix formed *in situ* from Kollodis' MAPTrix™ Angio Kit (100 μL/cm$^2$) using M199 media, and incubated at 37°C, 5% $CO_2$. Combinatorially activated integrin αvβ3-α2β1 (Fig 1A) and αvβ3-α5β1 (Fig 1B) can induce HUVEC tube formation under serum free conditions.

### Characteristics

MAPTrix™ Angio is produced in Kollodis' proprietary *E.coli* expression system and purified using an ISO compliant manufacturing process.

**Molecular Weight:**

- ~24,000 dalton

**Formula:**

- Lyophilized powder is also available upon request

**Solubility:**

- Soluble in a variety of buffers, including water, under a wide range of pH conditions (pH=2~9.0)
- Note: Buffers of media containing $Ca^{2+}$ or $Mg^{2+}$ added to MAPTrix™ may result in the formation of insoluble aggregates.

### Quality Control

| | |
|---|---|
| · Purity | 93% by SDS PAGE |
| · pH | 6.0 ~ 7.5 |
| · Endotoxin | Less than 20 EU/mL per LAL assay. |
| · Sterility | Tested and found negative for the presence of bacteria, fungi and mycoplasma before freezing dry |
| · Functionality | The biological activity of each peptide is determined in a cell culture assay under serum free conditions |

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

## Materials Supplied:

| Cat. No | Brand | Components |
|---|---|---|
| 370072 | MAPTrix™ Angio C<br>Targeting αvβ3 & α2β1 | - MAPTrix™ ECM, 10mg vial<br>· IKVAV (7.5mg) + GFPGER (2.5 mg)<br>- MAPTrix™ Linker: 50mg vial (4- & 8-arms PEG) |
| 370073 | MAPTrix™ Angio C<br>Targeting αvβ3 & α2β1 | - MAPTrix™ ECM, 20mg vial<br>· IKVAV (15mg) + GFPGER (5mg)<br>- MAPTrix™ Linker: 100mg vial (4- & 8-arms PEG) |
| 370074 | MAPTrix™ Angio C<br>Targeting αvβ3 & α2β1 | - MAPTrix™ ECM, 50mg vial<br>· IKVAV (37.5mg) + GFPGER (12.5mg)<br>- MAPTrix™ Linker: 200mg vial (4- & 8-arms PEG) |
| 370075 | MAPTrix™ Angio C<br>Targeting αvβ3 & α2β1 | - MAPTrix™ ECM, 100mg vial<br>· IKVAV (75mg) + GFPGER (25mg)<br>- MAPTrix™ Linker: 500mg vials (4- & 8-arms PEG) |
| 370082 | MAPTrix™ Angio F<br>Targeting αvβ3 & α5β1 | - MAPTrix™ ECM, 10mg vial<br>· IKVAV (2.5mg) + GRGDSP (7.5 mg)<br>- MAPTrix™ Linker: 50mg vial (4- & 8-arms PEG) |
| 370083 | MAPTrix™ Angio F<br>Targeting αvβ3 & α5β1 | - MAPTrix™ ECM, 20mg vial<br>· IKVAV (5mg) + GRGDSP (15mg)<br>- MAPTrix™ Linker: 100mg vial (4- & 8-arms PEG) |
| 370084 | MAPTrix™ Angio F<br>Targeting αvβ3 & α5β1 | - MAPTrix™ ECM, 50mg vial<br>· IKVAV (12.5mg) + GRGDSP (37.5mg)<br>- MAPTrix™ Linker: 200mg vial (4- & 8-arms PEG) |
| 370085 | MAPTrix™ Angio F<br>Targeting αvβ3 & α5β1 | - MAPTrix™ ECM, 100mg vial<br>· IKVAV (25mg) + GRGDSP (75mg)<br>- MAPTrix™ Linker: 500mg vials (4- & 8-arms PEG) |

## Storage Conditions:

- Kit materials can be stored at 2-8°C for up to 24 months.
- Once the MAPTrix™ ECM sample has been opened, store the remaining, unused powder at 2-8°C for 24 months. Remaining, unused solution of MAPTrix™ ECM can be stored at 2-8°C for 6 months. ***DO NOT FREEZE*** the remaining solution.
- Once the MAPTrix™ Linker has been opened, store the remaining, unused powder at -20°C for 6 months with appropriate sealing. ***DO NOT USE*** the remaining solution.



UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

## Protocols

This protocol describes how to make artificial basilar lamina in a 48-well plate format for tube formation assay. The protocol can easily be adapted for use with 6-, 12-, 24-, and 96-well plates.

### 1. Preparation of Hydrogel, Artificial Basement Membrane

- Dissolve MAPTrix™ Linker at a concentration of 20 mg in 1mL of PBS buffer, pH 7.4
- Dissolve MAPTrix™ ECM at a concentration of 20 mg in 1mL of PBS buffer, pH 7.4
- Vortex mix the two above solutions for 30 to 60 seconds. Alternatively, pipette up and down thoroughly to mix.

  **Note:** All MAPTrix™ solution should be filtered through a 0.22 µm filter prior to use

  *Once the MAPTrix™ Linker is added to MAPTrix™ ECM solution,* ***you have less than 20 minutes before the hydrogel forms*** *and it becomes impossible to pipette the solution*
- The prepared MAPTrix™ gelling solutions of at least 100µL per well were added to a 24-well plate and allowed to gel in a humidified incubator at 37 °C for 2~3 hours.

  **Note:** ***Pipette slowly to avoid air bubble formation in the gelling solution****. A 2 hour incubation is highly recommended. A fibrous surface morphology of in situ formed hydrogel may be obtained when the incubation is longer than 3 hours, resulting in a poor capillary tube formation or no tube formation at all.*
- The artificial basement membrane *in situ* formed is washed with 5 mL of M199 media for 30 minutes before its use. PBS buffer (1x) may be used as an alternative when M199 media is not available

① Dissolving

② Mixing

③ Transfer to wells

### 2. Endothelial tube formation assay

- Endothelial cells such as HUVECs are washed in serum-free wash medium by centrifuging at 400g for 5 min, and then the washed endothelial cells are seeded onto the surface of artificial basement membrane at a density of $4x10^4$ cells/$cm^2$ and incubated for 1 hour at 37 °C, 5% $CO_2$.
- On an as needed basis, endothelial growth media such as M199 media or growth factors such as VEGF may be supplied to the wells. Change the media if necessary.
- Incubate the tube formation assay plate for 16 to 24 hours at 37°C, 5% $CO_2$ atmosphere. The morphology of HUVECs may be monitored or imaged with a phase contrast microscope at regular intervals (every 6 hours is recommended)
- Measurement/Quantification of tube formation - Labeling with dyes such as BD Calcein AM Fluorescent Dye.

④ Cell seeding

⑤ Quantification

## References


1. D. G. Stupack and D. A. Cheresh. Integrins and Angiogenesis. Current Topics in Developmental Biology (2004) 64, 207-238
2. James J. Moon, et al., Vascularization of Engineered Tissues: Approaches to Promote Angiogenesis in Biomaterials. Current Topics in Medicinal Chemistry (2008) 8, 300-310
3. Irina Arnaoutova, et al., The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. Angiogenesis (2009) 12:267–274





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184 Park Drive, Milton Park
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F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85