

## Technical Data Sheet

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### MAPTriX HyGel™ Kit, mussel adhesive protein based adhesive hydrogel

#### Technical Information

##### Introduction

Synthetic hydrogels are appealing materials for biomedical applications since they offer higher lot-to-lot uniformity with a more controllable and reproducible scaffold structure, gel formation dynamics, degradation rates and mechanical properties. However, the lack of bioactivity or immunogenicity of synthetic polymer-based hydrogels has limited their widespread use in biomedical applications<sup>1</sup>.

Protein-g-PEG hydrogels that mimic *in vivo* environments, such as natural extracellular matrix environments, have been designed via a three-step process by using PEG diacrylate or PEG vinyl sulfone<sup>2</sup>.

MAPTriX HyGel™ provides a simpler, more reliable, and reproducible one-step process for the formation of hydrogels for biomedical and related applications.

##### Description and Applications

Multi-arm PEG derivatives have been used as cross-linking agents in a variety of ways for hydrogel formation. In particular, PEG-succinimidyl glutarate reacts predominantly with the lysine groups in mussel adhesive proteins. Cross-linking reactions amongst the multiple arms of the PEG derivative and the mussel adhesive protein results in a covalently bonded three-dimensional (3D) matrix.

Depending upon the reaction conditions, such as concentration or temperature, the physical characteristics of the hydrogel can exhibit quite different surface morphologies. For example, a sponge-like or a fibrous morphology can be formed that is suitable for 3D cell culture and related applications.

Highlighted features of using MAPTriX HyGel™ include:

- amenable to design and modification in order to customize specific bioactive and functional requirements
- exhibits no cytotoxicity or biocompatibility problems, all the while being chemically compatible with aqueous solutions, cell culture and physiological conditions
- compatible for microscopy and molecular biology analysis
- sufficiently stable for long time shelf-life and transportation

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**General Procedure****1. Solution Preparation:**

- Under aseptic conditions, place a lyophilized MAPTrix™ ECM powder in an e-tube and add an appropriate amount of buffer solution to the e-tube to dissolve the MAPTrix™ ECM powder; and then vortex mix for at least 2 minutes.
- Repeat for the MAPTrix™ Linker powder in order to make a 2 wt% of MAPTrix™ Linker solution.
- The recommended concentration of MAPTrix™ ECM and MAPTrix™ Linker solution should be at least 1 wt%; however optimal concentrations for your specific application should be determined (refer to the Surface Morphology Control section, below, for details).

**2. Mixing:**

- Under aseptic conditions, thoroughly mix an equal volume of MAPTrix™ ECM solution and MAPTrix™ Linker solution.
- Vortex mixing is highly recommended for thorough mixture of the solutions; but, mixing via pipette back-and-forth transfer in order to avoid trapping air bubbles, if applicable, is also recommended.

**3. Gelation:**

- As soon as possible (within 5 minutes of making the mixed solution of MAPTrix™ ECM and MAPTrix™ Linker), add 100 µL of the mixed solution to a 48-microwell plate in order to form the hydrogel.
- The recommended loading amount of MAPTrix HyGel™ for other microwell formats, such as 24-well, is summarized in Table 1 (below).
- Gelation usually starts within 5 minutes to 30 minutes if the MAPTrix™ ECM concentration is more than 20 mg/mL (refer to the gelation time with MAPTrix™ ECM concentration described in Surface Morphology Control, given below).

**4. Gelation Temperature:**

- Gelation temperature significantly influences the surface morphology of *in situ* hydrogel formation.
- Usually a highly porous matrix is generated when gelation occurs at room temperature while fibrous matrix is generated when gelation occurs at 37 °C (refer to the Surface Morphology Control for details, given below).

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Surface  
Morphology  
Control

The surface morphology of MAPTriX HyGel™ varies depending upon the gelation conditions as demonstrated in Figure 1 (below).

In general, the faster you allow gelation formation to occur, the more fibrous morphology you will get. However, a test run is highly recommended in order to optimize your experimental requirements.

- Standard Gelation Conditions to form 1mL of HyGel™
  - \* MAPTriX™ ECM: 2 wt% solution (10mg/0.5mL PBS)
  - \* MAPTriX™ Linker: 3 wt% solution (15mg/0.5mL PBS)
  - \* PBS (1X) as buffer solution used to dissolve the materials

All samples are added to the microwell plate within 5 minutes of preparing the mixed solution comprised of MAPTriX™ ECM and MAPTriX™ Linker; and then it is allowed to set for gelation for 3 hours under a given temperature condition. After about 5 minutes, the mixed solution becomes increasingly viscous.

Table 1. Standard concentration for *in situ* hydrogel formation

Case No.	MAPTriX™ ECM	MAPTriX™ Linker	Gelation Time	Gelation Temp	Morphology
1	2wt% 0.5 mL	4-arm 0.5 mL	5 min	25 °C	Homogeneous Sponge-like
2	2wt% 0.5mL	4-arm, 3wt% 0.5 mL	5 min	37 °C	Fibrous
3	2wt% 0.5 mL	6-arm 0.5 mL	20 min	25 °C	Sponge-like
4	2wt% 0.5 mL	6-arm 0.5 mL	20 min	37 C	Web-like fibrous

Table Notes:

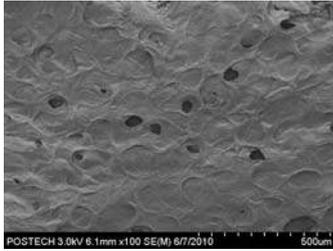
- Gelation time was defined as the moment of actual no flow due to the increased elastic modulus of the materials.
- If gelation time is critical for your experiment, a test run is highly recommended in order to adjust the concentration or the pH of the buffer (refer to the effect of pH on gelation time, below). Please take note, the concentration of MAPTriX™ ECM or MAPTriX™ Linker also influences the gelation time.

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- Surface Morphology Control

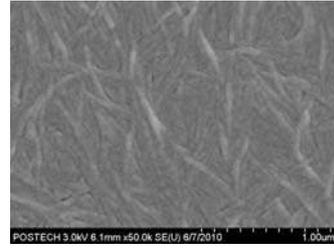
Figure 2. Surface morphology observed by SEM without cryo-facture

Case #1: HyGel formed at 25 °C



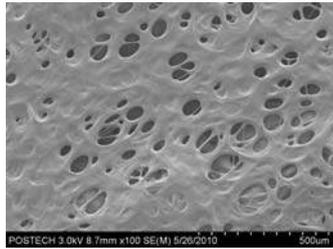
Homogeneous sponge-like surface morphology. The average pore size was approximately 100µm.

Case #2: HyGel formed at 37 °C



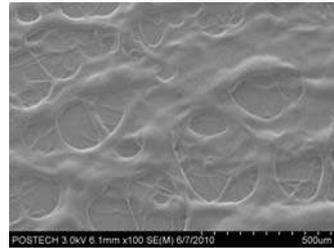
Nanofibrous surface morphology. The average diameter of fiber was approximately 60 nm.

Case #3: HyGel formed at 25 °C



Web-like surface morphology. The average diameter of fiber ranged from approximately 50µm to 100µm.

Case #4: HyGel formed at 37 °C



Web-like microscale fibrous surface morphology. The cross-section image showed a web-like fibrous morphology structure. (see below)

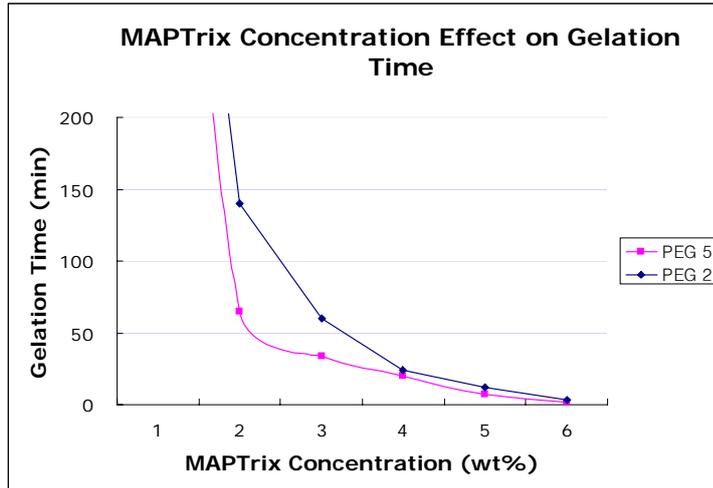
The concentration given above in Table 1 can be varied in several ways:

- The amount of MAPTrix™ ECM can be decreased down to 1 wt% when 4 arm PEG (MW=20,000) is used.
- The amount of MAPTrix™ Linker can be increased for a stiffer hydrogel formation.
- Other MAPTrix™ ECM products can be used in a combinatorial way in order to mimic the biochemical composition of natural basement-membrane.

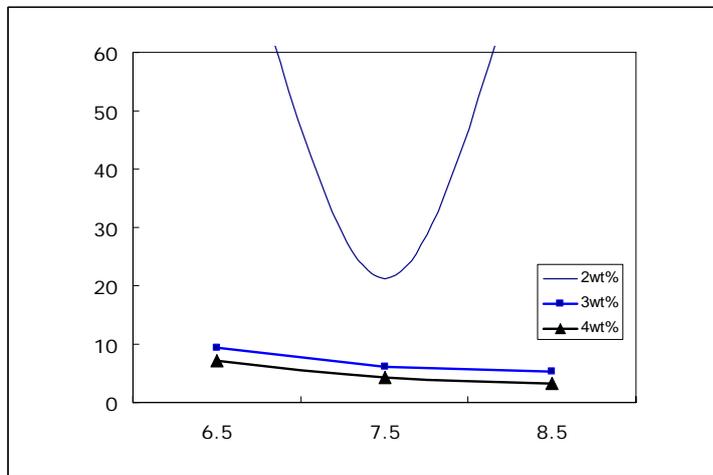
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Gelation Time Control

### 1. MAPTrix™ ECM Concentration effect on Gelation Time



### 2. pH effect on Gelation Time



Under weakly acidic conditions, for example pH=6.5 or lower, the gelation time increases; and, under basic conditions, gelation time shortens. If a longer gelation time is required, the pH of the buffer solution should be adjusted to weakly acidic conditions; however, do not adjust the pH lower than pH=5 otherwise PEG activity is significantly lost.

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## Loading Amount

- Suggested volumes of MAPTriX HyGel™ solution per well (volumes are based on using a standard concentration of 0.1mg/mL).

Table 2. Recommended loading amount of MAPTriX HyGel™

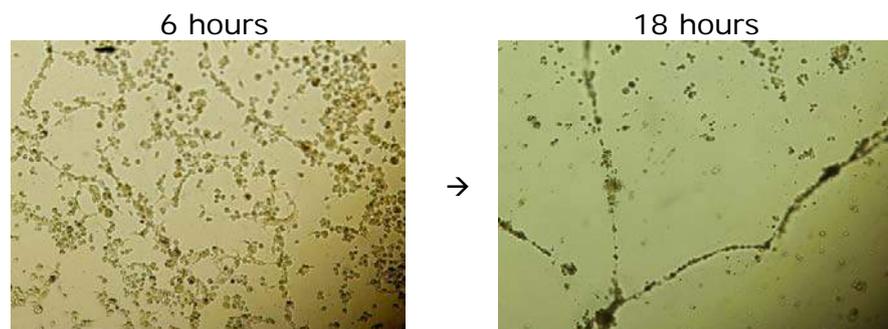
Culture ware	Spec.	Culture area (cm <sup>2</sup> /well)	MAPTriX HyGel™ Volume (mL/well)
Plates	6-well	9.6	1.20
	12-well	3.5	0.44
	24-well	1.9	0.24
	96-well	0.75	0.10
Dishes	35mm	8.8	1.10
	60mm	21.5	2.69
	100mm	56.7	7.09
Flasks	25	25	3.13
	80	80	10.00
	175	175	21.88

Table Note: The culture area calculated based on the NUNC brand of products

## Cell Culture Applications

### Endothelial tube formation

Endothelial growth media (M199 media), supplemented with 10% fetal bovine serum (FBS) and endothelial cell growth supplement (ECGS, 30 µg/ml; Sigma), was used to HUVEC cell culture.



HUVEC cells were seeded on MAPTriX HyGel™ (20,000 cells/cm<sup>2</sup>) and incubated in growing media. The tubule formation cultured on MAPTriX HyGel™ was evident after 1 day; and, tubule lengths and diameters varied greatly, from 20 to 50 µm, with an average of about 30 µm.

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Compared to a positive control of HUVEC tubule formation cultured on GelTrex™, the diameter of the tube formed on MAPTrix HyGel™ was larger.

The sizes and lengths of tubules appear to be dependent upon the porosity of the 3D matrix and pore size<sup>3</sup>. According to T.D. Dziubla *et al.*, the size of tubule seems to be directly proportional to the diameter of the pore size and matrix porosity. The volume average pore size and porosity for MAPTrix HyGel™ used for this tube formation assay are shown in Figure 2.3 (Case #3). The volume average pore size varied from 15 to 20 µm, markedly larger than those in GelTrex™, probably due to the large pore diameter of the MAPTrix HyGel™.

## References

1. Jindrich Kopecek, et al., Peptide-directed self-assembly of hydrogels. *Acta Biomaterials* 5 (2009) 805-816
2. N. Tirelli, et al., Poly(ethylene glycol) block copolymers, *Rev Mol Biotechnol* 90 (2002), 3-15
3. T.D. Dziubla et al., Vascularization of PEG-grafted macroporous hydrogel sponges: a three-dimensional in vitro angiogenesis model using human microvascular endothelial cells, *J. Biomed. Mater. Res.*, A68 (2004), p603-614

Information and Technical Assistance :

AMSBIO | [www.amsbio.com](http://www.amsbio.com) | [info@amsbio.com](mailto:info@amsbio.com)



**UK & Rest of the World**  
AMS Biotechnology (Europe) Ltd  
184 Park Drive, Milton Park  
Abingdon, UK  
T: +44 (0)1235 828 200  
F: +44 (0) 1235 820 482



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



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



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

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