TheWell BIOSCIENCE
Growing Cells in New Dimensions

PROTOCOL

VitroGel® Angiogenesis Assay Kit

CAT NO. VHM06-K1, VHM06-K2, VHM06-K3

- Multiple applications in one kit: Tube formation, invasion, and animal injection
- Control the hydrogel properties: Add your own growth factors and compare with positive control

Overview

VitroGel Angiogenesis Assay Kits are revolutionary tools for researchers to study the effects of both the hydrogel properties and culture medium on an angiogenesis process. The kits can be used to study the angiogenesis tube formation and invasion on both 2D hydrogel coating method and 3D cell culture method. The VitroGel Angiogenesis Assay systems are also good for animal injection for *in vivo* study.

Angiogenesis is a highly regulated process that involves the growth of new blood vessels from the existing vasculature. This process plays an important role in both normal developmental processes and numerous pathologies, including wound healing, tumor growth, and metastasis to inflammation and ocular disease.

Traditional angiogenesis assay highly relies on natural extracellular matrix (natural ECM), which has non-adjustable hydrogel compositions and properties. Therefore, our understanding of the angiogenesis process is limited by studying the molecular cues such as growth factors and inhibitors in culture medium only. There is a lack of knowledge on how the properties of hydrogel affect the angiogenesis process.

There are two versions of VitroGel Angiogenesis Assay Kits:

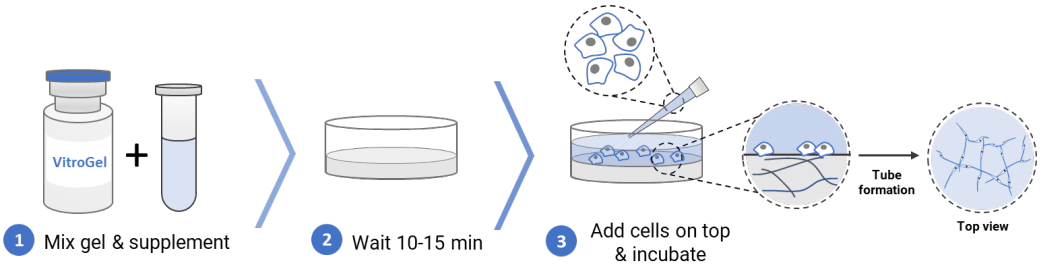
- VitroGel Angiogenesis Assay Kit (Cat No. VHM06-K): Ready-to-use with fixed hydrogel mechanical strength to support the angiogenesis assay with adjustable supplements.
- VitroGel Angiogenesis Assay HC Kit (Cat. No. TWG011-K): Assay kit with a tunable high concentration hydrogel to allow full control of the hydrogel's mechanical strength with adjustable supplements.

The ready-to-use VitroGel Angiogenesis Assay Kit contains:

- VitroGel AAK, a xeno-free ready-to-use hydrogel.
- AAK Supplement 1, a hydrogel growth supplement without vascular endothelial growth factors (VEGFs) for cell attachment and growth.
- AAK Supplement 2, a hydrogel tube formation supplement with VEGFs as a positive control for tube formation.

The VitroGel AAK hydrogel is room temperature stable and can be directly mixed with each supplements at 2:1 (v/v) ratio for hydrogel formation. Researchers can adjust the hydrogel's molecular cues by adding the growth factors/inhibitors directly to the supplements before mixing with the VitroGel AAK hydrogel. Cells cultured in this system can be further harvested easily with the VitroGel Cell Recovery Solution.

2D Hydrogel Coating Protocol



Recommended materials and reagents

- VitroGel® Angiogenesis Assay Kit
- Cells
- Cell culture medium
- Growth factors/inhibitors (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture plate

AAK Supplement 1 from VitroGel Angiogenesis Assay Kit is used as an example below. Replace AAK Supplement 1 with AAK Supplement 2 for tube formation assay.

1. Bring VitroGel AAK hydrogel to room temperature or warm at 37 °C.
2. Add 1mL VitroGel AAK hydrogel solution to 500 µL AAK Supplement 1 and gently pipette up and down 5-10 times to mix thoroughly. Note: Keep VitroGel and AAK Supplement 1 at 2:1 v/v mixing ratio.
   Optional: To control the critical growth factors/inhibitors in hydrogel, add desired growth factors/inhibitors in AAK Supplement 1 at 3X concentration. The modified supplement then can mix with VitroGel AAK hydrogel solution to get 1X final concentration).
3. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are

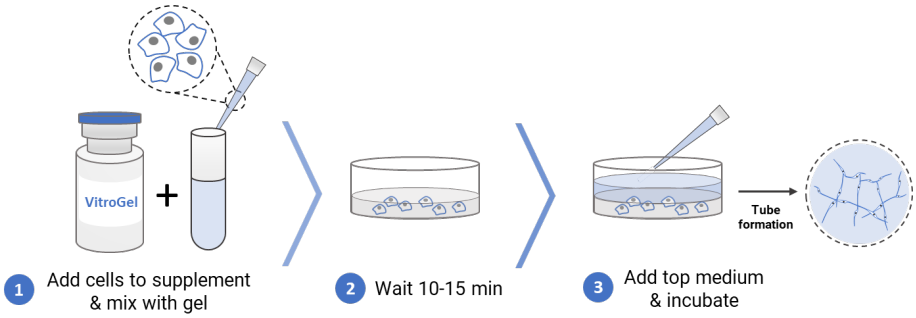
	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

4. Wait 10-15 min at room temperature for a soft gel formation.
   Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.
5. Carefully add medium with cells on top of hydrogel (Recommend cell concentration of 5 x 10^5 cells/mL). The

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

6. Place the well plate in an incubator. For long term culture, change the cover medium every 48 hours.
   Note: We recommend to only change 50-80% of the top medium without disturbing the hydrogel.

3D Cell Culture Protocol



Recommended materials and reagents

- VitroGel® Angiogenesis Assay Kit
- Cells
- Cell culture medium
- Growth factors/inhibitors (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture plate

AAK Supplement 1 from the VitroGel Angiogenesis Assay Kit is used as an example below. Replace AAK Supplement 1 with AAK Supplement 2 for tube formation assay.

1. Bring VitroGel AKK hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the AAK Supplement 1.
   - Recommended cell concentration 1-2 x 10^6 cells/mL.
   - Optional: To control the critical growth factors/inhibitors in hydrogel, add desired growth factors/inhibitors in AAK Supplement 1 at 3X concentration. The modified supplement then can mix with VitroGel AAK hydrogel solution to get 1X final concentration).
3. Add 1 mL VitroGel AAK hydrogel solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio).
4. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are list below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

5. Wait 10-15 min at room temperature for a soft gel formation. Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.
6. Carefully add additional medium to cover the hydrogel. The recommended volumes of cover medium for specific well plate types are list below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

7. Place the well plate in an incubator. For long term culture, change the cover medium every 48 hours. Note: We recommend to only change 50-80% of the top medium without disturbing the hydrogel.

Prepare Hydrogel for Animal Injection

AAK Supplement 1 from the VitroGel Angiogenesis Assay Kit is used as an example below. Replace AAK Supplement 1 with AAK Supplement 2 for tube formation assay.

1. Bring VitroGel AAK hydrogel to room temperature or warm at 37 °C.
2. Prepare cell suspension in the AAK Supplement 1.
   - Recommended cell concentration 1-2 x 10^6 cells/mL.
   - Optional: To control the critical growth factors/inhibitors in hydrogel, add desired growth factors/inhibitors in AAK Supplement 1 at 3X concentration. The modified supplement then can mix with VitroGel AAK hydrogel solution to get 1X final concentration).
3. Add 1 mL VitroGel AAK hydrogel solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio).
4. Transfer the hydrogel mixture to a syringe.
5. Let mixture stabilize at room temperature for 10-20 min. The hydrogel is ready for animal injection.

Protocol for Cell Recovery from VitroGel Angiogenesis Assay Kit

- For 3D cell culture and 2D hydrogel coating, refer to Protocol-1 of the VitroGel Cell Recovery Solution Protocol.