Setting up the angiogenesis assay

A. Prepare the complete ATCC® Angio-Ready™ Angiogenesis Medium
1. Thaw Angio-Ready™ Angiogenesis Medium (ATCC® ACS-2008B) in the refrigerator overnight.
2. Add 0.2 mL of rhVEGF (ATCC® PCS-999-024) to the Angiogenesis Medium.

B. Setting up the angiogenesis assay
1. Place the frozen cell vials in 37°C water bath for about 5 minutes; when thawed, transfer the cells to a 50 mL tube using a serological pipette. Add 10 mL of complete Angio-Ready™ Angiogenesis Medium, and pellet the cells at 150 X g for 4-6 minutes.
2. Carefully remove the medium using a 10 mL serological pipette, taking care not to disturb the cell pellet. Resuspend the cell pellet in 15 mL of fresh complete Angio-Ready™ Angiogenesis Medium by gently pipetting up and down with a 10 mL pipettor.
3. Immediately transfer the cell suspension to a 50 mL reagent reservoir.
4. Transfer 150 μL of the cell suspension to each well of the 96 plates using multi-channel pipet; avoid introducing bubbles to the cells.
5. Place the 96 well plate in a 37°C incubator with 5% CO₂ or a live cell imaging system.
6. Compound screening can begin within 18 hours post-seeding. It is recommended to run the assay in triplicate for each condition and to include a no compound control as one of the conditions. If a positive control is desired, please talk to ATCC technical service for your individual needs.
7. Change the complete medium every 2-3 days. Tubules should be visible within 72 hours after plating and will increase thereafter for the no compound control.
8. Analyze the results 7-8 days after the initial plating; the tubules can be analyzed live or fixed in 4% paraformaldehyde for additional markers staining.

References
For details regarding Angio-Ready Angiogenesis Medium with VEGF Supplement see ATCC® ACS-2008

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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