The Angio-*Ready*™ Assay System

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA

- World’s premiere biological materials resource and standards development organization

- ATCC collaborates with, and supports, the scientific community with industry-standard biological products and innovative solutions

- Strong team of 400+ employees; over one-third with advanced degrees
Agenda

Angio-Ready™ Assay System

- Background information
- Features, benefits, and applications
- Summary
Angiogenesis

The formation of new vessels from pre-existing vessels

Angiogenesis is an essential process during:

- Tissue development
- Wound repair
- Reproduction
- Tumor development
Angiogenesis

The first assay developed, still in wide use, was a gel matrix seeded with endothelial cells

- Human umbilical vein epithelial cells (HUVECs) were often used
- The tubules formed are short and relatively homogeneous
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- The tubules formed are short and relatively homogeneous
Angiogenesis – On a gel matrix – Live cell imaging
Advancement in angiogenesis assays

Co-culture of endothelial cells with fibroblasts

- Tubules are more heterogeneous, consisting of both short and long interconnecting tubules (capillaries)
- More representative of *in vivo* condition

The next step in getting to a more representative *in vivo*-like model
What is the Angio-Ready™ System?

A co-culture system consisting of telomerase-immortalized endothelial cells and mesenchymal stem cells, providing an *in vitro* angiogenesis system that is more close to the *in vivo* situation

- A mix of TeloHAEC-GFP (ATCC® CRL-4054™) and ASC52telo (ATCC® SCRC-4000™) provided with an optimized medium formulation
The TeloHAEC-GFP and hTERT-MSC co-culture system provides a real-time *in vitro* angiogenesis assay system.
## The Angio-*Ready™* System

<table>
<thead>
<tr>
<th>Product Description</th>
<th>ATCC® No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angio-<em>Ready™</em> Angiogenesis Assay System</td>
<td>ACS-2001-2™</td>
</tr>
<tr>
<td></td>
<td>(2 pack)</td>
</tr>
<tr>
<td>Angio-<em>Ready™</em> Angiogenesis Assay System</td>
<td>ACS-2001-10™</td>
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<tr>
<td></td>
<td>(10 pack)</td>
</tr>
<tr>
<td>Angio-<em>Ready™</em> Cells</td>
<td>ACS-2007™</td>
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<tr>
<td></td>
<td>(not available as stand-alone)</td>
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<tr>
<td>Angio-<em>Ready™</em> Angiogenesis Medium with VEGF Supplement</td>
<td>ACS-2008™</td>
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<tr>
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<td>200 mL</td>
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</tbody>
</table>

### 2 Pack
- 2 vials of Angio-*Ready™* cells (ATCC® ACS-2007™)
- 1 bottle of medium, 200 mL (ATCC® ACS-2008™)

### 10 Pack
- 10 vials of Angio-*Ready™* cells (ATCC® ACS-2007™)
- 5 bottles of medium, 200 mL (ATCC® ACS-2008™)
Assay overview

- Thaw and prepare Complete Angiogenesis Co-culture medium
- Thaw pre-mixed Angiogenesis Assay-Ready Cells (7.5 x 10^6 cells/vial)
- Wash, centrifuge, and resuspend cells in 15 mL Complete Co-culture Medium
- Pipette 150 μL of cell suspension into each well
- Screening can begin 18 hrs post-seeding
# Recommended cell seeding density for various culture vessels

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
<th>6-well plate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface area</strong></td>
<td>0.3 cm²</td>
<td>1.9 cm²</td>
<td>3.8 cm²</td>
<td>9.6 cm²</td>
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<tr>
<td><strong>Volume cell suspension to add</strong></td>
<td>150 µL</td>
<td>0.5 mL</td>
<td>1.0 mL</td>
<td>2.5 mL</td>
</tr>
<tr>
<td><strong>Total number of wells per vial</strong></td>
<td>96</td>
<td>16</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
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Applications

- Vessel formation in wound healing study and regenerative compound screening
- Angiogenesis inhibition study and inhibitors screening
- Angiogenesis under hypoxia situation study and compounds screening

Image courtesy of Lindsey Eldridge, Johns Hopkins University
Angiogenesis in the wound healing process

A. 12-24 hours post injury
   - Wound area is filled with blood clot
   - Neutrophils invade clot

B. 3-7 days post injury
   - Neutrophils undergo apoptosis
   - Macrophages are abundant
   - Endothelial cells proliferate and form new blood vessels
   - Fibroblasts deposit ECM
   - Keratinocytes proliferate and differentiate

C. 1-2 weeks post injury
   - Granulation tissue fills wound
   - Fibroblasts differentiate into myofibroblasts which deposit collagen and contract wound
   - Wound is covered with neoepidermis

The Angio-Ready™ System exhibits a dose-dependent response to VEGF.
The Angio-Ready™ System exhibits a dose-dependent response to VEGF.
Screening tumor angiogenesis inhibitors
Suramin blocks tubular structure growth in dose-dependent manner in the Angio-Ready™ System
Sunitinib blocks tubular structure growth in dose-dependent manner in the Angio-Ready™ System

TelohAEC-GFP and hTERT-MSC cells premixed, thawed and seeded immediately into wells of a 96-well plate, and treated with different doses of sunitinib; fixed and stained with anti-αSMA antibody at day 8
Sunitinib blocks tubular structure growth in teloHAEC-GFP and ACS52telo co-culture

Live images were recorded every 6 hours during an 8 day period
The Angio-Ready™ System exhibits a dose-dependent response to Bevacizumab inhibition.

Bevacizumab inhibits tubular growth

Tubular length (% of control) vs. Bevacizumab concentration, log(g/mL)
The Angio-Ready™ System demonstrates consistent lot-to-lot performance.
Hypoxia and tumor angiogenesis

a. Tumour cells grow along blood vessels
b. Increased tumour growth leads to hypoxia and necrosis
c. Angiogenic sprouting is initiated

Identification of approved and investigational drugs that inhibit hypoxia-inducible factor-1 signaling

Chia-Wen Hsu¹, Ruili Huang¹, Thai Khuc¹, David Shou¹, Joshua Bullock², Suzanne Grooby³, Sue Griffin⁴, Chaozhong Zou⁴, Annette Little⁵, Holly Astley⁶, Menghang Xia³

¹Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, USA

Figure 4: Anti-angiogenic properties of the identified HIF-1 inhibitors. (A) Fluorescence images of GFP-expressing aortic endothelial cells in the presence of various concentrations of mycophenolate mofetil. (B) Tube formation and viability of co-cultures of human aortic endothelial cells and human mesenchymal stem cells after a 3-day exposure of PI-103, trametinib, mycophenolate mofetil, and nielosamide under normoxic condition. Assay data are expressed as mean ± SD from three measurements.
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Summary

*In vitro* co-culture models provide a useful tool to examine angiogenesis

The tubules formed in co-culture assays more closely resemble capillaries than those formed on a gel matrix

Co-culture models utilizing primary cells suffer from:
- Donor variability,
- Low cell quantity per lot
- Short lifespan of primary cells

hTERT-immortalization eliminates donor variability

**Angio-Ready™ Angiogenesis Assay System**
- Can form tubular structures in < 7 days instead of 14 days compared to co-culture with fibroblasts
- Responds effectively to VEGF and drug treatment
- Immunofluorescence shows that cells surrounding the tubular structures stain positive for αSMA

These data support the physiological relevance of Angio-Ready™
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Please email additional questions to: tech@atcc.org