

CellPlayer™ Angiogenesis PrimeKit

Presentation, Storage and Stability

The Essen BioScience CellPlayer Angiogenesis PrimeKit is offered as either a live-cell kit (**Cat. # 4436**) or a cryopreserved kit (**Cat. # 4452**). The live-cell kit is composed of a tissue culture plate containing proliferating early passage HUVEC co-cultured with early passage NHDF in a specially formulated culture medium. The cryopreserved kit is composed of two boxes containing the reagents required to set up and run the angiogenesis tube formation assay to completion. Kit contents are as follows:

PrimeKit-Live (Cat. # 4436):

Room Temperature Shipment:

- 1) Sealed 96-well Assay Plate containing NHDF and HUVEC CytoLight Green in co-culture.
- 2) 5 x 25 mL bottles containing Assay Media
- 3) Sterile Plate Lid

Storage and stability:

- 1) Upon arrival, remove the seal on the assay plate and the place **IMMEDIATELY** at 37 °C. After acclimating to 37 °C, replace with fresh assay media containing growth factor and/or test agent.
- 2) The optimized assay media in this kit should be **REFRIGERATED** at 4 °C until use or the indicated expiration date. The kit contains sufficient culture media to support network development over the 14 day period.

PrimeKit-Cryo (Cat. # 4452)

Dry Ice Shipment:

- 1) Normal Human Dermal Fibroblast (NHDF) Cell Vial
- 2) Human Umbilical Vein Endothelial Cells expressing CytoLight Green (HUVEC CytoLight Green)
- 3) Seeding Media Supplement (2 mL total volume)
- 4) Growth Media Supplement (0.4 mL total volume)
- 5) Assay Media Supplement (2.5 mL total volume)

Room Temperature Shipment:

- 1) Seeding Basal Media (40 mL total volume)
- 2) Growth Basal Media (20 mL total volume)
- 3) Assay Basal Media (125mL total volume)
- 4) 96-well Assay Plate

Storage and stability:

- 1) Upon arrival, the cells in this kit should be **IMMEDIATELY** transferred to liquid nitrogen. When stored in liquid nitrogen, they will remain viable until the indicated expiration date.
- 2) The media supplements contained in this kit should be kept **FROZEN** at -20 °C until use or expiration date.
- 3) The basal media in this kit should be **REFRIGERATED** at 4 °C until use or the expiration date located on each bottle. The kit contains sufficient culture media to support network development over the 8-10 day period.



Background

The CellPlayer Angiogenesis PrimeKit contains early passage normal human endothelial cells and early passage normal human interstitial cells. When handled according to the Essen BioScience CellPlayer Angiogenesis protocol, this co-culture model recapitulates all phases of the *in vivo* angiogenesis process, including cell proliferation, migration, morphogenesis, and anastomosis. Under basal conditions, these cultures exhibit a low level of capillary-like tubules after 8-14 days, unless stimulated with a growth factor such as VEGF, EGF, or bFGF. Tubule development is believed to closely mimic *in vivo* angiogenesis and is enhanced by known angiogenic stimulators. Measuring anti-angiogenic events requires the presence of a stimulating factor, such as VEGF. Both enhancement and suppression occur in a concentration-dependent manner.

Recommended Usage / Quality Control Testing

The CellPlayer Angiogenesis PrimeKit contains matched cells and all media components required to complete a successful experiment. Our 3 years of experimental development has repeatedly shown that growth factors purchased from different sources can result in highly variable responses. As such, all growth factor supplement and inhibitor kits available for purchase from Essen BioScience, including VEGF, bFGF, EGF, and suramin have been rigorously quality controlled to ensure maximal tube formation and/or inhibition if used as described. When used in conjunction with the IncuCyte-FLR, the resulting effect of growth factors and test agents on tube formation can be measured using the Essen BioScience automated angiogenesis algorithm. The data generated with this algorithm can be used to assess angiogenic potential via tube length, branch point, and tube area metrics.

Extensive testing of the CellPlayer Angiogenesis PrimeKit is performed to ensure a significant level of growth factor-mediated tube formation compared to the untreated control. Utilizing historical data generated across multiple experiments at Essen BioScience, we typically observe average tube length levels of 9.8 ± 1.1 mm/mm² (Mean \pm SD) when using VEGF at 4ng/ml. Addition of 100 μ M suramin will inhibit VEGF-mediated tube formation by > 90%. Careful adherence to the PrimeKit protocol will minimize unwanted edge effects, enabling the use of the entire 96-well plate for test agent investigation. Our strict criteria for assay performance enable high-quality assessment of a test agents' pro- or anti-angiogenic potential in an *in vitro* setting.

Related Products

Cat.# 4453 CellPlayer Cryo-preserved HUVEC CytoLight Green

Cat.# 4437 CellPlayer VEGF/Suramin Supplement Kit

Cat.# 4438 CellPlayer bFGF/Suramin Supplement Kit

Cat.# 4439 CellPlayer EGF/Suramin Supplement Kit

Cat.# 4541 CellPlayer Angiogenesis Optimized Media Kit

For additional information including a simplified description of the protocol and an extensive application note, please visit our webpage at http://www.essenbioscience.com/angiogenesis_primekit.html.

For a detailed protocol including use of the CellPlayer™ Angiogenesis PrimeKit, as well as additional information, please contact Essen BioScience at: sales@essenbio.com.

This Essen BioScience product contains proprietary nucleic acid(s) coding for proprietary fluorescent protein(s) being, including its derivatives or modifications, the subject of pending patent applications and/or patents owned by Evrogen





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