Bespoke GFP Angiogenesis Assay Protocol

Equipment

37°C 5% CO_2 Incubator
Glass Haemocytometer for manual cell counting
Light Microscope
Laminar Flow Hood
Serological pipettes
Tissue Culture Flasks

Materials and Reagents:

Cells		**Storage Temp.**
ZHC-2402	GFP HUVEC, Angiogenesis tested, 0.5 x 10^6 cells/vial	LN_2
ZHC-5102	Human Dermal Fibroblasts (HDFa), Angiogenesis tested, 1 x 10^6 cells/vial	LN_2
ZHM-5923	**Human Fibroblast Growth Medium (FGM)**	
KC1017	Human Fibroblast Basal Medium, 500ml	2-8°C
KC1018	Human Fibroblast Growth Medium Supplement	-20°C
KC1019	Antibiotic Supplement	-20°C
ZHM-2953	**Human Large Vessel Endothelial Cell Growth Medium (LVGM)**	
KC1015	Human Large Vessel Endothelial Cell Basal Medium, 500ml	2-8°C
KC1014	Growth Supplement, 10ml	-20°C
KC1019	Antibiotic Supplement	-20°C
ZHA-1960 &ZHA-1970	**Angiogenesis Seeding (ASM) and Growth Medium (AGM)**	
KC1012	Angiogenesis Basal Medium, 500ml	2-8°C
KC1013	Angiogenesis Seeding Medium Supplement, 25ml	-20°C
KC1014	Growth Supplement, 10ml	-20°C
KC1019	Antibiotic Supplement	-20°C
ZHA-1300	**Angiogenesis Assay Controls**	
KC1007	Suramin Control Compound (1mM)	2-8°C or -20°C
KC1006	VEGF Control Compound (2µg/ml)	2-8°C or -20°C

Trypsin 1 x solution	SIGMA
Trypsin/EDTA	Sciencell
Buffered saline rinsing solution	Sciencell
Trypsin blocking solution	Sciencell
Trypan Blue	SIGMA

Method

Cell culture in the Laminar Flow Hood should be undertaken in strict sterile conditions.

Day 1

1. Prepare a bottle of Human Fibroblast Growth Medium (FGM) by supplementing Human Fibroblast Basal Medium with Human Fibroblast Growth Medium Supplement then equilibrating it to 37°C in a 5% CO_2 incubator for 30 minutes.
2. Prepare a bottle of Human Large Vessel Endothelial Cell Growth Medium (LVGM) by supplementing Human Large Vessel Endothelial Cell Basal Medium with Growth Supplement then equilibrating it to 37°C in a 5% CO_2 incubator for 30 minutes.
3. Thaw the HDFa and HUVEC cells in a water bath.
4. Count the cells under a light microscope using a haemocytometer and the Trypan Blue viability stain.
5. In the laminar flow hood, add the contents of the ampoules into tissue culture flasks containing the relevant medium i.e. FGM for HDFa and LVGM for HUVEC, and place flasks in the 5% CO_2 incubator thereby seeding the cells. Cells should be seeded such that both cell lines are able to be harvested at the same time when less than confluent.

Day 2

6. After 24 hours, remove the flasks from the incubator and check the cells for growth and sterility under the microscope.
7. In the laminar flow hood, aspirate the medium and replace with fresh FGM and LVGM.
8. Return flasks to the 5% CO_2 incubator.
9. Continue steps 6 to 8 until cells are nearing confluency. The medium will only need changing after the initial change and then every 2/3 days.

Day X

10. Prepare a bottle of Angiogenesis Seeding Medium by supplementing Angiogenesis Basal Medium with Angiogenesis Seeding Medium Supplement then equilibrating it to 37°C in a 5% CO_2 incubator for 30 minutes.
11. Before HDFa and HUVEC are confluent, trypsinise the cells according to the V2a Kit protocol.
12. Count cells as in step 4.
13. Plate cells in a 24 well plate into Angiogenesis Seeding Medium at a ratio that promotes suitable endothelial cell tubule formation.
14. Return plate to the 5% CO_2 incubator for 24 hours.

Day X+1

15. Prepare a bottle of Angiogenesis Growth Medium by supplementing Angiogenesis Basal Medium with Growth Supplement then equilibrating it to 37°C in a 5% CO_2 incubator for 30 minutes.
16. In the laminar flow hood, aspirate the medium and replace with Angiogenesis Growth Medium, containing control (Suramin and VEGF) and test compounds.
17. Return plate to the 5% CO_2 incubator.
18. Continue to change the medium (containing control and test compounds) every two to three days until 14 days have elapsed.

Day X+14

19. Proceed to quantify tubule formation using your chosen technique. Alternatively, Cellworks offer an Angiogenesis Image Analysis Service (Product Code ZHA-6000). Customer reports include the number of tubules, the number of junctions, the total tubule length, and the mean tubule length for each image. Please contact cellworks@caltagmedsystems.co.uk for advice about using this service prior to running the assay.

Cellworks, a division of Caltag Medsystems Ltd
● Whiteleaf Business Centre ● 11 Little Balmer ● Buckingham ● MK18 1TF ● UK
T: +44 (0) 1280 827460 **F:** +44 (0) 1280 827466 **E:** office@caltagmedsystems.co.uk **W:** www.cellworks.co.uk
VAT Reg. No. GB 765 6661 89 Registered in the UK No. 4162330