Datasheet for ABIN2344899

Endothelial Tube Formation Assay (In Vitro Angiogenesis Assay)

36 Publications

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Overview

Quantity:	50 tests
Application:	Biochemical Assay (BCA)

Product Details

Sample Type:	Cell Samples
Detection Method:	Fluorometric
Characteristics:	Endothelial Tube Formation Assay Kit utilizes ECM (extracellular matrix) gel prepared from Engelbreth-Holm-Swarm (EHS) tumor cells. Endothelial tube formation on ECM gel closely mimics the in vivo environment and may be used to test angiogenesis inhibitors before in vivo testing. Endothelial Tube Formation Assay Kit provides a robust system to assess angiogenesis in vitro. Tube formation can be achieved within 18 hours. Following staining the tube with the provided fluorescence dye, the extent of tube formation, such as average tube length and branch point, can be quantified through imaging software. Each kit provides sufficient quantities to perform up to 50 assays in 96-well plates.
Components:	1. ECM Gel Solution : Five 0.5 mL tubes, Ready-To-Use 2. 10X Staining Buffer : One 1 mL tube 3. Staining Dye : One 50 µL tube of 1 mM Calcein AM in DMSO
Material not included:	1. Endothelial cells such as HUVEC, HMEC 2. Endothelial cell culture medium 3. 1XPBS 4. 37 °C Incubator, 5 % CO2 atmosphere 2 5. Light microscope 6. Fluorescence microscope 7. (optional) Tube quantification software

Target Details

Background:	Angiogenesis, or neovascularization, is the process of generating new blood vessels derived as extensions from the existing vasculature. The principal cells involved are endothelial cells, which line all blood vessels and constitute virtually the entirety of capillaries. Angiogenesis involves multiple steps, to achieve new blood vessel formation, endothelial cells must first escape from their stable location by breaking through the basement membrane. Once this is achieved, endothelial cells migrate toward an angiogenic stimulus such as might be released from tumor cells or wound-associated macrophages. In addition, endothelial cells proliferate to provide the necessary number of cells for making a new vessel. Subsequent to this proliferation, the new outgrowth of endothelial cells needs to reorganize into a three-dimensionally tubular structure. Each of these elements, basement membrane disruption, cell migration, cell proliferation, and tube formation, can be a target for intervention, and each can be tested in vitro and in vivo. Several in vivo assay systems, including the chick chorioallantoic membrane (CAM) assay, an in vivo Matrigel plug assay, and the corneal angiogenesis assay, have been developed that permit a more realistic appraisal of the angiogenic response. However, these assays are time consuming, tedious and require technical specialties. One quick assessment of angiogenesis is the measurement of the ability of endothelial cells to form three-dimensional structures (tube formation). Endothelial cells form tubes on collagen or fibrin clot coated dishes after several days.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Assesses angiogenic tube formation in vitro • Uses an ECM matrix gel • Resembles in vivo basement membrane environment
Reagent Preparation:	• ECM gel: Thaw overnight on ice or in a frost-free 4 °C refrigerator, store the thawed ECM gel solution up to 10 days, do not refreeze. The ECM gel solution will gel rapidly at room temperature. The tube MUST be kept on ice all the time. • 1X Staining Buffer: Prepare a 1X Staining Buffer by diluting the provided 10X stock 1:10 in dH2O. Store the diluted solution at room temperature. • 1X Staining Solution: FRESHLY prepare 1X Staining Buffer by diluting the provided Staining Dye to 1 µM with 1X Staining Buffer. Store 1X Staining Solution at room temperature.
Assay Procedure:	I. Tube Formation 1. Thaw ECM gel and prepare 1X Staining Buffer as described above. 2. Add 50 µL of thawed ECM gel solution to each well of a pre-chilled 96-well sterile plate. Note: Undiluted ECM gel solution is very viscous and quickly gels at room temperature, so it MUST

Application Details

be kept on ice all the time. When pipetting, use a pre-chilled pipette tip to prevent any ECM gel formation during transfer.

3. Incubate 30 minutes to 1 hr at 37 °C to allow the ECM solution to form a gel.
4. Harvest endothelial cells and resuspend in desired culture medium containing 0.5-10 % serum and your desired angiogenesis mediators at 1-2 x 105 cells/mL.
5. Add 150 µLof cell suspension (1.5-3 x 104 cells) per well onto the solidified ECM gel. Incubate the assay plate at 37 °C for 4 to 18 hrs.
6. Examine the endothelial tubes using light microscope in high magnification field. It is possible to visually estimate the extent of tube formation by inspecting the overall tube length and branch points.

II. Fluorescence Labeling

1. Carefully remove medium by gently blotting on paper towels. Be carefully not to disturb endothelial tubes.
2. Gently wash with 100 µL of 1X Staining Buffer and remove the wash as described in step
    1. 3. Add 50 µL of 1X Staining Solution per well and incubate 30 min at 37 °C.
    2. Gently wash with 100 µL of 1X PBS and remove the wash as described in step
        1. Repeat this step twice. 3
        2. Examine endothelial cells and tubes using a fluorescence microscope. Acquire several images per well and process them using imaging analysis software such as NIH Image or Image Pro Plus. Note: Calcein AM-labeled tubular structure is stable for only 1-2 hrs in 1X PBS at 4 °C

Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	Store all components at -20°C.

Publications

Product cited in:

Sakaguchi, Katagiri, Osugi, Kawai, Sugimura-Wakayama, Hibi: "Periodontal tissue regeneration using the cytokine cocktail mimicking secretomes in the conditioned media from human mesenchymal stem cells." in: **Biochemical and biophysical research communications**, Vol. 484 , Issue 1, pp. 100-106, (2017) (PubMed).

Katagiri, Sakaguchi, Kawai, Wakayama, Osugi, Hibi: "A defined mix of cytokines mimics conditioned medium from cultures of bone marrow-derived mesenchymal stem cells and elicits bone regeneration." in: **Cell proliferation**, Vol. 50, Issue 3, (2017) (PubMed).

Kim, Kim, Auh, Yi, Moon, Kim: "Role of Protein Phosphatase 1 in Angiogenesis and Odontoblastic Differentiation of Human Dental Pulp Cells." in: **Journal of endodontics**, Vol. 43, Issue 3, pp. 417-424, (2017) (PubMed).

Sun, Wang, Li, Qian, Shen: "Forkhead box protein k1 recruits TET1 to act as a tumor suppressor and is associated with MRI detection." in: **Japanese journal of clinical oncology**, (2016) (PubMed).

Chang, Kim, Kim, Kim, Yi, Lee, Kum, Kim: "Combined effects of mineral trioxide aggregate and human placental extract on rat pulp tissue and growth, differentiation and angiogenesis in human dental pulp cells." in: **Acta odontologica Scandinavica**, Vol. 74, Issue 4, pp. 298-306, (2016) (PubMed).

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