

BD BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation

Frequently Asked Questions

What are the parameters measured in the BD BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation product?

The BD BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation measures the extent of endothelial cell tube length as an indicator of endothelial cell tube formation. While various researchers have measured a number of parameters such as tube length, tube area, and branch points, we obtained consistent and reproducible results based upon the measurement of tube length alone.

Do I need special equipment to perform the assay?

Not necessarily. Either an automated image acquisition instrument or a fluorescence microscope, which is capable of taking images/pictures, can be used. An automated image acquisition instrument provides rapid data collection and data processing, which enables you to read and process the entire plate at one time. If an automated image acquisition instrument is not available, you can use a fluorescent microscope to take pictures of each well, and process the images using image-processing software. In our assay, we use an automated image acquisition instrument (Gen I Cell Based Screening System) and data processing software (MetaMorph®), both are manufactured by Universal Imaging Corporation™, www.image1.com.

Note: Data generated is based upon using the Gen I Cell Based Screening System and MetaMorph software, specifically configured for quantification of tube length.

Do I need special software to calculate my results?

Yes. This assay requires software that is capable of image processing. In our assay, quantification of endothelial cell tube formation is performed using MetaMorph software manufactured by Universal Imaging Corporation to process images of fluorescently labeled tubes. Some other commonly used image processing software packages include Image-Pro (MediaCybernetics®, www.mediacy.com/mediahm.htm) and NIH Image, <http://rsb.info.nih.gov/nih-image/>).

Note: If you need assistance configuring your software to quantify tube length, please contact the software manufacturer's technical or sales representative.

What types of endothelial cells can I use in the BD BioCoat Angiogenesis Tube Formation assay?

Each lot of BD BioCoat Angiogenesis System: Endothelial Cell Tube Formation is tested with HMEC-1 cells. We also successfully used HUVEC and HMVEC cells. Other types of endothelial cells may be used, however, you will need to optimize the conditions of these cells prior to use.

How long can I let the assay incubate? Can I incubate for 24 hours?

It is important not to let the cells incubate longer than 16-18 hours. Prolonged incubation has no detrimental effect on tube formation per se. Longer incubation, however, may result in the tubes being washed off during the labeling process.

Are tiny bubbles on the edges of the wells normal?

It is normal to have some small bubbles on the edges of the wells. They generally fall outside of the acquisition field when the wells are centered under the microscope. Occasionally you may only see a part of the bubble in the microscopic field. If this is the case, the interference and impact upon the results is minimal.

Can I stack plates during thawing or incubation?

No. The plates must not be stacked during thawing or short incubations. Stacked plates do not thaw or warm evenly or at the same speed, which may lead to inconsistent assay results among plates and among assays.

Can I store the plates in a frost-free freezer or -70°C freezer?

No. The plates should be stored at -20°C. Storing the plates in either a frost-free or a -70°C freezer will result in product stability issues, which will affect overall product performance.

I observe broken tubes after Calcein AM staining but the tube networks were intact before labeling. How can I avoid this?

This can be avoided by very gently decanting either the assay medium or wash buffer followed by using a paper towel to gently blot the remaining liquid.

I did not use all 96 wells in my experiment. Can I use the remaining wells in a subsequent assay?

No. Once the BD Matrigel™ Matrix gels and your assay is completed, the plate cannot be reused and must be discarded.

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