BioResearch

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Protocol for Performing a Trypan Blue Viability Test

Technical Reference Guide

This protocol describes how to perform a Trypan Blue staining which can be used to discriminate between viable and non-viable cells.

- Dilute your cell sample in Trypan Blue dye of an acid azo exclusion medium by preparing a 1:1 dilution of the cell suspension using a 0.4% Trypan Blue solution. Non-viable cells will be blue, viable cells will be unstained. Trypan Blue should be sterile filtered before using it in order to get rid of particles in the solution that would disturb the counting process.

- Carefully and continuously fill the hemocytometer chamber.

- Incubate the hemocytometer and cells for 1 – 2 minutes at room temperature. For longer incubations, please use a humid chamber. Incubations exceeding 30 minutes may cause decreased cell viability due to Trypan toxicity.

- Count cells under the microscope in four 1 x 1 mm squares of one chamber and determine the average number of cells per square (all hemocytometers consist of two chambers, each is divided into nine 1 mm^2 squares). For an accurate determination, the total number of cells overlying one 1 mm^2 should be between 20 – 50 cells/square. If the cell density is higher than 200 cells/square, you should dilute your cell suspension. For information on hemocytometers including a protocol for the Neubauer chamber, please see the associated Technical Reference Guide “Cell Counting and Determination of Viability via Hemocytometer”.

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