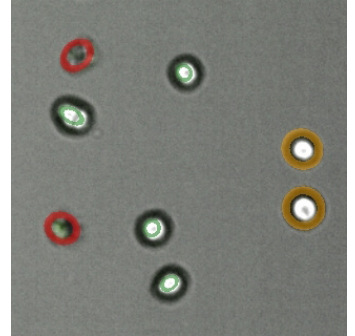


Trypan Blue (AnxV)

GENERAL PURPOSE

This application is a combination of the common used Trypan Blue viability test with an Annexin V (FITC labeled) conjugate as a probe to detect phosphatidylserine (PS) on the cell surface. The location of PS on the outer surface is found in an early apoptotic status of cells as well as other forms of cell dead.



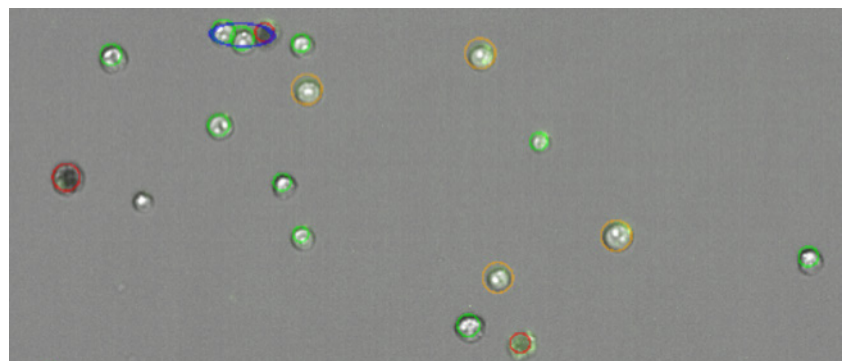
RESULT TABLE

Viability	Percentage of viable cells in the sample
VCD	Viable Cell Density [#/ml]
CD	Cell Density [#/ml]
Apoptotic Cells	Number of cells that are apoptotic
Apoptotic Ratio	Percentage ratio of apoptotic cells
Cell Count	Number of cells listed per well
Avg Cell Size	Average of the cell size [μm^2]
# of Aggregates	Number of aggregates
Aggregates per ml	Number of aggregates per ml
Ratio of Aggregates	Percentage ratio of aggregates in the sample
Reactor ID	Name of the reactor, entered as plate layout
Sample ID	Name of the sample, entered as plate layout
Final Dilution	Dilution factor, entered as plate layout
Volume per Well	Sample volume per well, entered as plate layout

EXAMPLE

This example shows a typical result image of a Trypan Blue Annexin V (FITC labeled) assay.

- Marked green:** Viable cells
- Marked red:** Dead cells
- Marked orange:** Apoptotic cells
- Marked blue:** Aggregated cells



DILUTION TABLE

The following table refers to the Corning Costar Half Area plate (# 3695) and an Annexin V-FITC conjugate that is supplied by Sigma Aldrich (A9210-10UG).

Expected Cell Density	Final Dilution [1]	PBS ⁻ [μl]	Binding Buffer / AnxV-FITC [μl]	Sample [μl]	TryB 0,02% [μl]	
1x10 E7	⇒⇒	1:80	760	18.9 / 1.1	20	800
5x10 E6	⇒⇒	1:40	360	18.9 / 1.1	20	400
1x10 E6	⇒⇒	1:20	160	18.9 / 1.1	20	200
5x10 E5	⇒⇒	1:10	60	18.9 / 1.1	20	100
1x10 E5	⇒⇒	1:5	20	18.9 / 1.1	20	40
less than 1x10 E5	⇒⇒	1:4	0	18.9 / 1.1	20	40

PLATE LAYOUT

Plate Layout Configuration

Group Configuration
 Group Name Start Count

SubGroup Configuration
 Wells SubGroup Count
 Replicates Horizontal
 Replicates Vertical
 Numbering Direction

Group Properties

Name	Start	Direction	Step	Physical Unit
Reactor ID	1	▶	0	1
Sample ID	1	▶	0	1
Final dilution	1:40	▶	1:1	1
Volume per Well	40	▶	0	μl

To obtain results such as the cell density in the result table, the software needs to know what volume and what dilution was used in for each well. These two details can be entered in the "Prepare" tab with "Layout" and well selection.

In addition, it is possible to give your samples a name which appears in the result table.