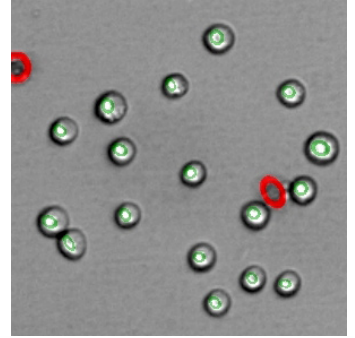


# Trypan Blue

## GENERAL PURPOSE

A common method to monitor a culture run is a viability test by using Trypan Blue. Customary systems will usually take a sample volume of about 300  $\mu\text{L}$  to 600  $\mu\text{L}$  to evaluate the sample status which takes about 2 to 3 minutes. SYNENTEC's Trypan Blue assay approach requires a sample volume of 20  $\mu\text{L}$  only which is scanned in less than 2 seconds. No major consumable expenses reduce the cost per sample to \$ 0.05 and less.



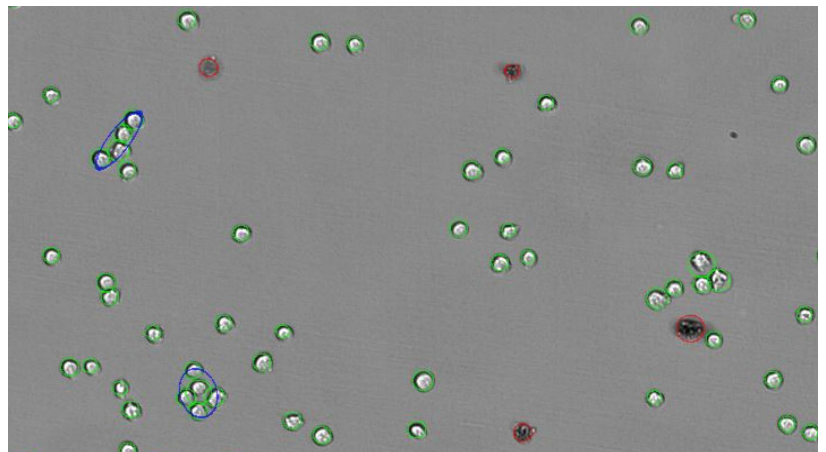
## RESULT TABLE

Viability	Percentage of viable cells in the sample
VCD	Viable Cell Density [#/ml]
CD	Cell Density [#/ml]
Cell Count	Number of cells listed per well
Avg Cell Size	Average of the cell size [ $\mu\text{m}^2$ ]
Aggregates per ml	Number of aggregates per ml
Ratio of Aggregates	Percentage ratio of aggregates in the sample
# of Aggregates	Number of aggregates
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 analysis.

- Marked green:** Viable cells
- Marked red:** Dead cells
- Marked blue:** Aggregated cell



## DILUTION TABLE

The following table shows the recommended dilution of the cell sample in relation to the expected cell density based on a Corning Costar half area plate (\*cat # 3695; 40 µL/well suggested). **We recommend never taking less than 20 µL cell suspension/dilution!** Otherwise the method error will increase significantly (data not shown). **In this table the number of possible replicates per approach varies. If more replicates are desired, please scale up the approach accordingly.**

Expected Cell Density		Final Dilution [1]	PBS <sup>-</sup> [µL]	Sample [µL]	TryB 0.02% [µL]
$1 \cdot 10^7$	→	1:80	780	20	800
$5 \cdot 10^6$	→	1:40	380	20	400
$1 \cdot 10^6$	→	1:20	180	20	200
$5 \cdot 10^5$	→	1:10	80	20	100
$1 \cdot 10^5$	→	1:5	30	20	50
Less than $1 \cdot 10^5$	→	1:2	0	20	20

## PLATE LAYOUT

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.