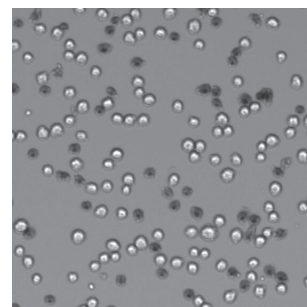


Erythrosin B

GENERAL PURPOSE

The **Erythrosin B** image analysis application of YT-SOFTWARE® is used to determine viable cell counts. The principle underlying the use of Erythrosin B is based on membrane permeability: viable cells exclude the dye due to their intact membranes, whereas non-viable cells, which have compromised membranes, take up the dye and appear red or dark under brightfield microscopy. The application calculates several parameters, including the *viability*, *cell count*, *cell density*, and *viable cell density*.



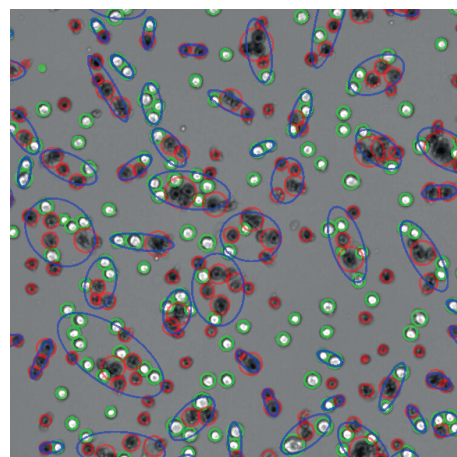
RESULT TABLE

Viability	Percentage of viable cells [%]
VCD	Viable cell density [#/mL]
CD	Cell density [#/mL]
Cell Count	Total cell count per well [#]
Avg Cell Size	Average of the cell size [μm^2]
Aggregates per mL	Number of aggregates per mL [#/mL]
Ratio of Aggregates	Percentage ratio of aggregates in the sample [%]
# of Aggregates	Number of aggregates [#]
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 [μL], entered as plate layout

EXAMPLE

This example shows CHO-K1 cells stained with 0.005 % (w/v) erythrosin B and analyzed with the **Erythrosin B** image analysis application.

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



DILUTION TABLE

We recommend adjusting the final cell concentration (after the addition of erythrosin B) to approximately 3×10^5 cells/mL, as this represents the optimal range for accurate measurement. If the initial cell suspension is expected to contain a substantially higher concentration, perform a pre-dilution in a microtube. The following table shows exemplary volumes for 96-well half-area plates. These volumes are calculated to provide a final sample volume sufficient for at least three technical replicates per sample (40 µL/well). We recommend using no less than 20 µL of cell suspension per well. Volumes below this threshold significantly increase method error.

Expected initial cell density	Final dilution	PBS [µL]	Sample [µL]	Erythrosin B [µL]
1×10^7	1:80	780	20	800
1×10^6	1:40	380	20	400
1×10^6	1:20	180	20	200
5×10^5	1:10	80	20	100
1×10^5	1:5	60	40	100
Less than 1×10^5	1:2	0	60	60

PLATE LAYOUT

Plate Layout Configuration X

GROUP CONFIGURATION

Group Name Start Count

SUBGROUP CONFIGURATION

	Wells	SubGroup Count
Replicates Horizontal	1	1
Replicates Vertical	1	1

Numbering Direction

GROUP PROPERTIES

NAME	START	DIRECTION	STEP	PHYSICAL UNIT
Sample ID	1	▶	0	1
Concentration	10	▶	0	µg/ml
Dilution	1:10	▶	1:1	1
Volume per Well	100	▶	0	
		▼		

For calculation of the *Cell Density/Viable Cell Density* the software needs to know the dilution and volume of the cell suspension that was used for each well. In addition, it is possible to give your samples a name which appears in the result table. These details can be entered in the *Prepare* tab with *Layout* and well selection.