VivaFix™ Cell Viability Assay

Catalog #	Description	Catalog #	Description
135-1111	**VivaFix 353/442 Cell Viability Assay**	135-1115	**VivaFix 498/521 Cell Viability Assay**
135-1112	**VivaFix 410/450 Cell Viability Assay**	135-1116	**VivaFix 547/573 Cell Viability Assay**
135-1113	**VivaFix 408/512 Cell Viability Assay**	135-1117	**VivaFix 583/603 Cell Viability Assay**
135-1114	**VivaFix 398/550 Cell Viability Assay**	135-1118	**VivaFix 649/660 Cell Viability Assay**

For research purposes only.

Description

VivaFix Cell Viability Assays are easy-to-use, versatile solutions for assessing the viability of mammalian cells by flow cytometry and microscopy. In most flow cytometry experiments, the exclusion of dead cells within a sample is an important step to prevent erroneous interpretation of the data caused by nonspecific fluorescence signals. When performing cell imaging experiments, cell viability and live:dead ratio are parameters that are frequently evaluated to measure cell health.

The proprietary dyes in the VivaFix Cell Viability Assay can easily assist researchers with distinguishing between live and dead cells by covalently binding to primary amines. In live cells, the VivaFix Dyes bind to the cell surface primary amines. In dead cells, where the plasma membrane is compromised, the dyes are able to permeate the cell and also react with intracellular primary amines. As a result, a greater number of fluorophores is associated with dead cells and at least a 100-fold difference in fluorescence intensity is measured between the live and the dead cells, thereby allowing an easier discrimination between the two populations (Figure 1). VivaFix Cell Viability Assays are available in a wide range of excitation and emission spectra, and can be combined into any multicolor experiment. VivaFix Dyes are compatible with cell fixation without any significant loss of fluorescence.

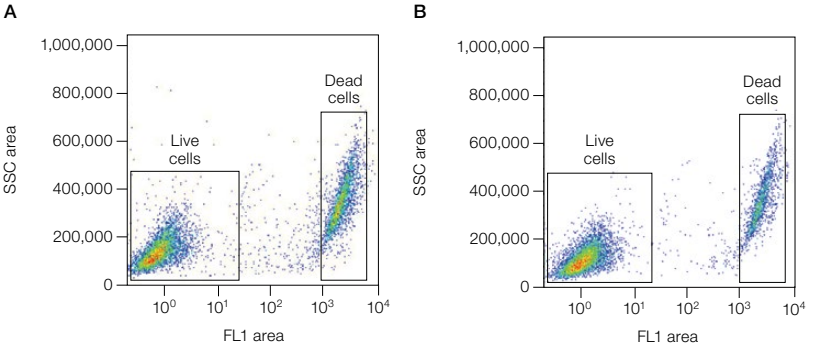

Fig. 1. Excellent separation between live and dead cells using the VivaFix Cell Viability Assay. Jurkat cells were stained with the VivaFix 498/521 Cell Viability Assay, fixed with 3.7% formaldehyde (**A**) or not fixed (**B**), and analyzed with the S3™ Cell Sorter. SSC, side scatter.

Kit Contents and Storage

Follow the guidelines in Table 1 for storing kit components.

Table 1. Kit components and storage.

Kit Component	Quantity	Storage, °C
VivaFix Viability Dye	4 vials (50 tests per vial)	–20
DMSO	1 vial (250 µl)	–20

Fixable Cell Viability Assays

Use Table 2 to select the appropriate VivaFix Cell Viability Assay to stain cells.

Table 2. Optimal excitation laser specifications.

Catalog Number	Labeling Dye Description	Optimal Excitation Laser, nm	S3 or S3e™ 488/561 nm Filter Channel	Compatibility with ZOE™ Fluorescent Cell Imager
135-1111	VivaFix 353/442	355	—	Blue channel
135-1112	VivaFix 410/450	405	—	—
135-1113	VivaFix 408/512	405	—	—
135-1114	VivaFix 398/550	405	—	—
135-1115	VivaFix 498/521	488	FL1 (525/30)	Green channel
135-1116	VivaFix 547/573	561	FL2 (586/25)	Red channel
135-1117	VivaFix 583/603	561	FL3 (615/25)	Red channel
135-1118	VivaFix 649/660	640	—	—

Assay Protocol

Important: Thaw all components prior to use.

Note 1: Any buffer without sodium azide, serum, or protein can be used instead of phosphate buffered saline (PBS).

Note 2: For multicolor experiments, the cells can be stained with the antibodies of your choice before or after staining with VivaFix Dye. Antibody staining conditions should be optimized for your assay.

1. Prepare a 500x stock solution by adding 50 µl of dimethyl sulfoxide (DMSO) to a VivaFix Dye vial and mix by vortexing.
2. Wash cells once with PBS, then resuspend cells at 2–3 x 10^6 cells/ml in PBS. Use 0.5 ml of cell suspension per assay.
3. Add 1 µl of 500x dye stock solution (from step 1) to 0.5 ml cells/assay and mix by vortexing.
4. Incubate the mixture for 30 min at room temperature (RT) or in a 37°C/5% CO_2 incubator. Protect from light.

Note: The optimal dye concentration and incubation time for different cell lines should be assessed empirically.

5. Wash cells twice in PBS.
6. Fix cells as desired. For 3.7% formaldehyde:
   — Suspend cells in 900 µl of PBS following step 5
   — Add 100 µl of 37% formaldehyde to the cell suspension
   — Incubate for 15 min at RT, then wash cells twice with PBS
7. Resuspend cells in the appropriate flow analysis buffer.
8. Sort cells with an S3 or S3e Cell Sorter, analyze cells with a flow cytometer, view and capture an image of cells with the ZOE Fluorescent Cell Imager, or view cells with a conventional fluorescence microscope.

Visit bio-rad.com/web/vivafix1 for more information.

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