Rev.: 001 Issued December 2014
Read this package insert carefully before use

REF **05-6000-01**

Yeast Control – Cell Cycle

INTENDED USE

This kit contains ready-to-use staining solutions for the fluorescent staining of yeast cells. The protocol requires ethanol fixation of the cells prior to staining. Yeast DNA staining is required for quantification of total DNA and yeast cell cycle analysis. The distribution of G1 and G2/M phases of the cell cycle varies during the fermentation of the yeast cells.

Stained samples can be analysed on a flow cytometer.

KIT COMPONENTS

Packing contains reagents for 50 tests:

- 5 x 10 mg RNase A/Solution A
- 4 ml Solution B

INSTRUCTIONS

For instrument alignment and quality control, please refer to the IFU of your Flow Cytometer.

Preparation of RNase A stock solution:

- Add 2.0 ml dest. H_2O to 1 tube RNase A/ Solution A
- Mix well

Store RNase A stock solution at -20°C

Preparation and staining of samples:

Fixation of yeast cells:

- 1 ml cell suspension from growing yeast cultures or bio reactors (at a cell density of approx. 5 x 10^6 cells/ml) are removed and washed twice with 5 ml PBS

  Remark: Spin-down of cells should be performed in a bench-top centrifuge at 5.000 x g for 5 minutes.

- After the last washing step cells are resuspended in 5 ml of ice cold 70% ethanol and stored for at least 12 hours at 4°C.

Shorter incubation times (but at least one hour) are possible with a potential loss of signal resolution. Incubation times with ethanol should be optimized for each yeast strain. Ethanolic cell suspensions may be kept at 2-8°C for several weeks.

Staining of fixed yeast cells:

- 1 ml of the ethanolic yeast cell suspension are washed twice with PBS
- after the last washing step cells are resuspended in 1 ml PBS and place into a sample tube (Code No. 04-2000)
- add 200 µl RNase A stock solution to the cells and incubate for at least 60 min at room temperature
- add 80 µl of *Solution B* to the cells and incubate for at least 60 min at room temperature protected from light
- analyze on a flow cytometer

Instrument requirements:

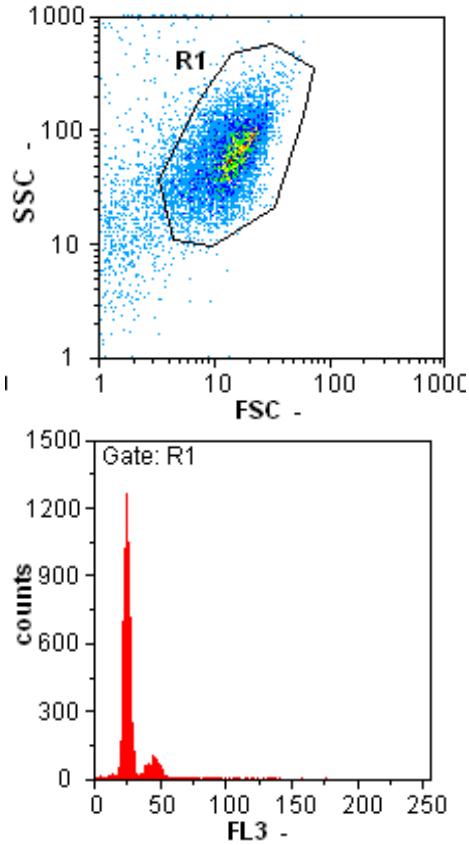
A flow cytometer equipped with blue laser light excitation (488 nm), capable of analyzing forward scatter (FSC), side scatter (SSC) and red fluorescence detecting parameter.

Instrument settings:

- Laser light source: 488 nm
- Trigger: FSC
- Red fluorescence: linear scale
- Speed: 0.5 µl/sec (or higher for low cell concentrations)

Data analysis:

- the yeast cell population will be presented in a dot plot of FSC – SSC
- define a region around the yeast cell population (R1)
- DNA distribution of fluorescently labelled cells can be shown in a histogram of the red fluorescence channel (FL3). Apply the selected gate R1 to this histogram.



Yeast suspension prepared with Yeast Control - Cell Cycle kit and measured on a CyFlow® SL

STORAGE AND STABILITY

Storage:	2-8°C in the dark
Shelf life:	please refer to the expiry date labeled on the bottle.

DISPOSAL PROCEDURE

Disposal procedure should meet requirements of applicable local regulations.

MANUFACTURER

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