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

CyStain® UV Ploidy REF 05-5001

INTENDED USE

CyStain® UV Ploidy is a ready-to-use staining solution for analyzing genome size variation and ploidy level of plant cells. It can be used for the fluorescence staining of nuclear DNA from plant tissues and of fixed and non-fixed cells from different origin. The prepared samples can be analyzed on flow cytometers with UV excitation and blue emission.

KIT COMPONENTS

Packing contains reagents for 250 tests:

- 500 ml *Staining Solution*

INSTRUCTIONS

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

Sample staining of fresh plant tissues:

• put about 5 mm^2 leaf tissue or other plant material in a petri dish (Order No.: 04-2005)
• add 0.5 ml *Staining Solution*
• chop the plant sample by using a **sharp** razor blade for 30 – 60 seconds [Razor blades need to be exchanged after 5-10 samples]
• add 1.5 ml *Staining Solution*
• incubate for 5 minutes at room temperature in the dark
• filter sample through 50 µm *CellTrics®* filter (Order No.: 04-0042-2317) into a sample tube (Order No.: 04-2000) and start analysing

Sample staining of fixed and non-fixed suspended cells:

Sample fixation (where required):
• spin cells from suspension culture down
•remove supernatant and wash once with PBS
•spin cells down again and remove PBS
•add 70% ice-cold EtOH and leave for at least 12 hours for fixation at 4 °C

Sample staining:
•spin about 2 x 10^6 cells down, choose conditions adequate for your cells
• wash the pellet with PBS or TRIS buffer (pH 7.0 - 7.5)
• spin cells down again; choose conditions adequate for your cells
• remove supernatant
• add 2.0 ml of *Staining Solution* to the pellet, vortex and incubate 5 minutes at room temperature in the dark - incubation over night at 4°C increases the homogeneity of the staining and therefore the resolution of analysis
• filter the sample through a 50 µm *CellTrics®* filter and start analysing

Fixation and staining of yeast cells

Sample fixation:
• harvest about 2 x 10^7 cells/ml
• spin the cells down, remove supernatant
• fix with 70% EtOH final concentration for at least 10 hours
• samples may be stored long termed at 4°C

Sample staining:
• spin the cells down, remove supernatant
• wash in PBS
• spin the cells down, re-suspend in 2 ml PBS
• dilute *Staining Solution* 3:1 with distilled water
• add 10 µl of diluted *Staining Solution* to 2 ml of fixed and washed yeast cells (1 x 10^7 cells/ml) in PBS
• incubate at least 10 minutes at room temperature in the dark (longer incubation improves results, i.e. for 10 hours in the dark at 4°C)
• after incubation start analysing

Instrument requirements:

A flow cytometer with UV excitation (λ = 355 nm – 375 nm) and a parameter for blue fluorescence emission (λ = 435 nm - 500 nm).

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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