

CyStain® PI Absolute P**REF 05-5022**

INTENDED USE

CyStain® PI Absolute P is a reagent kit for nuclei extraction and DNA staining of nuclear DNA from different plant species and tissues in order to determine absolute or relative genome size and ploidy level. It is a staining protocol for the fluorescent staining of nuclear DNA of fixed and non-fixed cells from different origin. The kit was developed for the use in cells obtained from cell suspensions and solid tissues, respectively. The procedure may include fixation with 70 % ethanol. Prepared samples can be analyzed on standard flow cytometers.

KIT COMPONENTS

Packing contains reagents for 250 tests:

- 125 ml *Nuclei Extraction Buffer*
- 500 ml *Staining Buffer*
- 2 x 1.5 ml *Propidium Iodide*
- 1 x 5 mg *RNAse A*

INSTRUCTIONS

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

Preparation of RNAseA stock solution:

- add 1.5 ml H₂O to 1 tube *RNAse A* [containing 5 mg RNAse A]
- mix well

Store RNAse A stock solution at -20°C

Preparation of staining solution for 10 samples:

- add to 20 ml *Staining Buffer* 120 µl *Propidium Iodide* and 60 µl RNAseA stock solution.

Stored at 2-8°C and protected from light the freshly prepared staining solution is stable for 24 hours.

Preparation and staining of samples:

- put approximately 0.5 cm² (or less) of leaf tissue or other plant material in a plastic petri dish (Code No.: 04-2005)
- add 500 µl *Nuclei Extraction Buffer*

- chop the sample by using a **sharp** razor blade for 30 – 60 seconds [Razor blades need to be exchanged after 5-10 samples]
- incubate for 30 -90 seconds
- filter sample through 50 µm *CellTrics®* filter (Order No.: 04-0042-2317) into a sample tube (Order No.: 04-2000)
- add 2 ml staining solution (= *Staining Buffer* + *Propidium Iodide* + RNAseA stock solution)
- incubate for 30 - 60 minutes protected from light at room temperature
- start analysing

Prepared samples are stable for about 12 h stored at 2-8°C in the dark.

Incubation with staining solution for some hours may improve the result. If samples oxidize add PVP (1%) or mercaptoethanol to the ready to use staining solution.

Instrument requirements:

A flow cytometer with 488 nm or 532 nm laser light source and a parameter of orange - red fluorescence emission (> 590 nm).

PRECAUTIONS

Propidium Iodide is toxic and carcinogenic. Handle only with protective clothing; please refer to MSDS.

STORAGE AND STABILITY

Storage: 2-8°C in the dark

Shelf life: Please refer to the expiry date, labeled on the bottle

RNAseA stock solution has to be stored at -20°C.

Prepared staining solution has to be stored at 2-8°C in the dark and is stable for 24 hours only.

DISPOSAL PROCEDURE

Disposal procedure should meet requirements of applicable local regulations.

MANUFACTURER

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