Rev.: 001 Issued July 2014

Read this package insert carefully before use

CyStain® PI Absolute T REF 05-5023

INTENDED USE

CyStain® PI Absolute T is a reagent kit for nuclei extraction and DNA staining of nuclear DNA from different species and a variety of tissues from human animal and plants, in order to determine absolute or relative genome size and ploidy level. This is also a staining protocol for the fluorescent staining of nuclear DNA of fixed and non-fixed cells from different origin, like cell suspensions and solid tissues, respectively. The procedure may include fixation with 70 % ethanol.

Prepared samples can be analyzed on standard flow cytometers.

CyStain® PI Absolute T is for use in basic research with no intended medical purpose as specified in MEDDEV.2.14/2 rev.1. It is therefore labeled as "for research use only" (RUO) product.

KIT COMPONENTS

Packing contains reagents for 250 tests:

- 125 ml *Nuclei Extraction Buffer*
- 500 ml *Staining Buffer*
- 5 x 0.525 g *Buffer Reagent*
- 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 ready to use extraction buffer:

Mix one tube *Buffer Reagent* (0.525 g) with 25 ml *Nuclei Extraction Buffer* in a separate flask. This mixture is the ready to use extraction buffer and is stable for maximal 7 days, stored at 2-8°C in the dark.

Preparation of RNase A stock solution:

- Add 1.5 ml H_2O 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 RNase A stock solution.

Stored at 18-25°C and protected from light the freshly prepared staining solution is stable for 24 hours.

Preparation and staining of samples:

Sample staining of fresh tissues:

- put about 50 mg plant tissue or less than 10 mg of animal or human tissue in a petri dish (Order No.: 04-2005)
- add 500 µl ready to use 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 2 -15 minutes at room temperature
- 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* + RNase A stock solution)
- incubate for 30 - 60 minutes protected from light at room temperature
- start analyzing

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.

Sample staining of fixed cells:

Cell fixation method:

Cells from suspension cultures and other suspensions like chicken and trout erythrocytes and human leukocytes are harvested without dispersing pre-treatment and are fixed with 70% ethanol.

Cells from bone marrow and other suitable tissues are isolated by flushing with EDTA containing Ca and Mg- free PBS. The cell suspension is then fixed with 70% ethanol.

Solid tissues are minced and incubated in the ready to use extraction buffer for 20 minutes at room temperature. The resulting single nuclei suspension is subsequently fixed with 70% ethanol.

All fixed cells can be stored at 2-8°C or –20°C as long as several months.

Cell staining procedure:

The fixed cells are spinned down at 200 g for 10 minutes and the fixative is removed completely. Cells from suspension cultures and other cells which have

not been treated with the ready to use extraction buffer before are re-suspended in one volume ready to use extraction buffer and are incubated for 15 minutes with gentle shaking. Subsequently five volumes of staining solution (containing PI and RNase A) are added and sample is prepared for analyzing on a flow cytometer.

Cells from solid tissues are re-suspended in one volume of ready to use extraction buffer and incubated for 10 minutes at room temperature with gentle shaking. Subsequently five volumes of staining solution (containing PI and RNase A) are added.
Incubate for at least 30 to 60 minutes, protected from light before analyzing on a flow cytometer.

The stained cells are stable for 24 – 48 hours in the dark.

Instrument requirements:
A flow cytometer with 488 nm or 532 nm laser light source and a parameter for 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:	18-25°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.
Staining solution (= *Staining Buffer* + *Propidium Iodide* + RNase A stock solution) has to be stored at 18-25°C in the dark and is stable for 24 hours only.

DISPOSAL PROCEDURE
Disposal procedure should meet requirements of applicable local regulations.

MANUFACTURER
Sysmex Partec GmbH
Am Flugplatz 13
02828 Görlitz
Germany