# Polyploidy assessment in plants using Flow Cytometry

***05-5022 CyStain® PI Absolute P***
*05-5022_Product Data Sheet_Rev 005_2011-08-11*
*Partec GmbH, D-48161 Münster, Germany*

## Principle

CyStain PI absolute P is a reagent kit for nuclei extraction and DNA staining of nuclear DNA from plant tissues in order to determine absolute or relative genome size and ploidy level. CyStain PI absolute P is well suited for the preparation of nuclear DNA staining of plenty of different plant species and a variety of different tissues. Samples can be analyses with standard flow cytometers with 488, 514 or 532 nm laser excitation or with mercury arc lamp flow cytometers using green excitation.

## Equipment and Reagents

| **Machine/Product** | **Reference (Company, Type, …)** |
|---|---|
| Crushed Ice | |
| CyStain® PI Absolute P Kit<br>*This kit contains:*<br>• *Propidium Iodide (PI) stock solution*<br>• *RNase stock*<br>• *Staining Buffer solution*<br>• *Extraction Buffer solution* |  Partec 05-5022 CyStain® PI Absolute P |
| Razorblades | |
| 55 mM plastic Petri dishes | |
| CellTrics 50µm filters | Partec 04-0042-2317 50 µm CellTrics Filters |
| 3.5 ml test tubes (12x75 mm) | |
| Pipettes | |
| Flow Cytometer | Accuri C-6 |

## Preparing RNase stock solution:

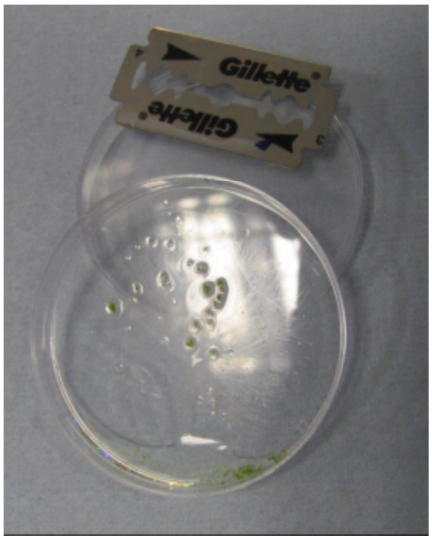
- **RNase stock solution:** Add 1.5 ml $H_2O$ to 5 mg RNase (Eppendorf tube) and mix well.
- **Staining solution:** Mix 2.0 ml Staining Buffer per sample with 12 µl of PI stock solution and 6 µl of RNase stock solution. E.g. to prepare 10, mix 20 ml of Staining Buffer with 120 µl of PI stock solution and 60 µl of RNase Stock solution. The staining solution with PI is stable for one day.
- Storage of the reagents:

| | |
|---|---|
| Extraction Buffer | 4°C |
| Staining Buffer | 4°C |
| PI Stock Solution | 4°C (protected from light) |
| RNase stock solution | -20°C (allow to warm up to RT before use.) |

**GENERAL NOTE**: If using samples with little plant material (like Lemna, or just one Arabidopsis leaf), you can use 250 µl Extraction buffer and stain with 1.0 ml staining buffer to obtain the same results.

## Nuclei isolation and staining procedure:

- Due to the big variations, make sure to have at least 6 replicates for each sample.




- Put approximately 0.5 cm² (or less) of leaf tissue or other plant material in a 55 mM plastic Petri dish
- Add 500 µl Extraction buffer
- Chop the material using a sharp razor blade for 30 to 60 seconds. Do not over-chop! 2 samples can be done with each side of a razor blade.
- After chopping, incline the Petri dish and collect all plant material and solution to one side using a pipette

***NOTE***: Chopping yields better results when using a "*used / scratched"* Petri dish: a dish where the cutting surface has been scratched and indented with a razor blade repeatedly.

- Leave the sample for 30 - 90 seconds of incubation in the extraction buffer (During this waiting time, the next sample can already be chopped)
- Rinse the plate again with the extraction buffer using a pipette.
- Transfer the sample to a Partec 50 µm CellTrics disposable filter with a pipette.
- Add 2.0 ml Staining solution (with PI and RNAse) to the test tube.
- Incubate on ice and in the dark for at least 30 to 60 minutes.
- Samples are stable for at least 12 hours at 4°C. Store stained samples at 4°C protected from light. Staining for several hours may improve the result. If samples oxidize add PVP (1%) or mercaptoethanol.

*CellTrics® 50µm, sterile, single packed*

**Polyploidy assessment by flow cytometry**

- Analyze in your flow cytometer in the red channel.
- Nuclei size is compared to PI intensity.
- Settings strongly depend on the used flow cytometer. Following procedure is specifically for the Accuri C-6 flow cytometer:

*Before running samples:*

1) Place an Empty 12x75mm tube on the SIP.
2) Click BACKFLUSH.
3) Place a ***fresh*** tube with 2 ml of filtered, DI $H_2O$ on the SIP.
4) Set **Time Limit** for 2 minutes and **Fluidics Speed** to FAST.
5) Click RUN.
6) Once the time limit is reached, Click DELETE SAMPLE DATA.
7) Remove the tube and run samples.

*Measuring a batch of samples:*

1) Click File - open file or template.
2) Select "Lemna template" or "Arabidopsis template."
3) Set **Time Limit** for 4 minutes, click RUN.

4) When the sum of events in the regions of interest (P1, P2, P3, P4,...) reach 100, the measurement can be stopped.

*After Running Samples:*

1) Place a tube with 2 ml of diluted cleaning solution (#KR-225) on the SIP.
2) Select an ampty data well.
3) Set **Time Limit** for 2 minutes and **Fluidics Speed** to FAST.
4) Click RUN.
5) Once the time limit is reached, remove the tube with cleaning solution.
6) Place a tube with 2 ml of filtered, DI $H_2O$ on the SIP.
7) Set **Time Limit** for 2 minutes .
8) Click RUN. The C-6 will stop automatically when the time limit is reached.
9) Leave the tube on the SIP until the C-6 is used again.
10) Push the ON/OFF button for no more than 1 second to shut it down.

## Analysis of the results:

After exporting the results to Excel, the relative percentages of the counts targeted zones (P1, P2, P3,...) containing the different polyploidy levels (2n, 4n, 8n, ...) are compared with each other.