Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

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1. Identification

Product name **PI/ Cell Cycle Analysis Kit**
200 rxn

Cat. No: **CA112**

2. Description

Cell cycle is a ubiquitous, complex sequence of events leading to growth and proliferation of cells. Cell cycle of most eukaryotic cells is divided into four discrete phases: M, G1, S, and G2. S phase is the period during which DNA replication occurs. The cell grows throughout interphase, which includes G1, S, and G2.
Cells at different stages of the cell cycle can also be distinguished by their DNA content. The DNA content of cells duplicates during the S phase (2n to 4n) and DNA content then remains at 4n for cells in G2 and M, decreasing to 2n after cytokinesis.
Experimentally, cellular DNA content can be determined by incubation of cells with a fluorescent dye that binds to DNA, followed by analysis of the fluorescence intensity of individual cells in a flow cytometer. The fluorescence signal is proportional to cellular DNA content.
Propidium iodide is a fluorescent molecule that binds nucleic acid with little or no sequence preference. Because Propidium iodide binds RNA as well as DNA, RNase A is included in this kit to digest cellular RNA and thus decrease background RNA staining from the experiment.

3. Composition

Item	Quantity
Propidium Iodide (PI) Reagent	2 mL
10x Phosphate Buffered Saline	50 mL
RNase A Solution	2 mL

! Caution: Propidium iodide is a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper protective clothing and gloves when handling the solution.

4. Storage specifications

Upon receipt, store RNase A solution at -20˚C. Store the PI Reagent and 10X PBS at +4˚C. Protect PI solution from light.

5. Applications

- ✓ Analysis of cell cycle regulation in response to external stimuli, environmental changes, etc.
- ✓ Monitoring of cell cycle progression.
- ✓ Study effects of drugs which affect cell growth and division.

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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7. Working solutions

- **1X PBS:** Dilute 10X Phosphate Buffered Saline 1:10 to make a 1x working solution.
- **Staining Solution:** Immediately before use, prepare the Propidium Iodide + RNase A Solution in 1X PBS. *For example, for every 20 samples to be stained, mix 10 mL 1XPBS + 200 µL Propidium Iodide + 200 µL RNase A.*
  Protect from light. Stable for one week at 4˚C

8. Assay Procedure

A. Sample Preparation:

1. Grow cells of interest (2-5 x 10^5 cells/well).
2. Generate a single cell suspension in a manner similar to routine passaging of the cells.
3. Harvest cells and centrifuge at 500 x g for 5 min. Aspirate and discard the supernatant.
4. Wash cells in 1 ml ice cold 1X PBS.
5. Centrifuge cells at 500 x g for 5 min. Aspirate and discard the supernatant.

B. Nucleic Acid Labelling:

6. Fix the cells by adding 1.2 ml ice cold 70 % ethanol to the cell pellet. Add drop wise to the pellet while vortexing. Put on ice for at least 30 min.
   After fixing, cells can be stored at -20˚C for several weeks in 70% ethanol.
7. Centrifuge cells at 500 x g for 5 min. and carefully remove the supernatant without disrupting the pellet.
8. Wash cells in 1 ml of 1X PBS.
9. Centrifuge cells at 500 x g for 5 min. and carefully remove the supernatant.
10. Resuspend cells completely with 200 µl of Staining Solution, protect from light exposure.
11. Incubate at 37 ºC for 30 min.
12. Place tubes on ice (still in the dark) and prepare for flow cytometry analysis.

C. Data Analysis:

13. Select the main cell population in the FSC vs SSC plot to exclude debris and cell aggregates.
14. Collect propidium iodide fluorescence in the appropriate channel (typically FL2).
15. Setting up markers on a histogram plot to delineate 4N intensity regions facilitates quantifying differences in DNA content between samples within an experiment.

