Miltenyi Biotec

Live Cell Discrimination Kit

Order no. 130-097-378

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1. Description

Components	**2 mL Hoechst 33342 Solution (1.5 mg/mL)**
	2 mL Propidium Iodide Solution (100 µg/mL).
Capacity	200 tests or up to 2×10^8 total cells.
Product format	The Hoechst 33342 Solution is supplied in distilled water at a concentration of 1500 µg/mL. The Propidium Iodide Solution is supplied in phosphate-buffered saline at a concentration of 100 µg/mL.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Hoechst 33342 is a cell permeable nucleic acid dye that intercalates in A-T regions of DNA. Therefore the staining intensity differs between different cell types. All nucleated cells are Hoechst positive.

Propidium iodide (PI) is a fluorescent dye that intercalates into double-stranded nucleic acid. It is excluded from viable cells, but can penetrate cell membranes of dead or dying cells.

1.2 Applications

- Exclusion of dead cells and enumeration of viable nucleated cells.

1.3 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant VYB (# 130-096-116), with a 488 nm laser for the excitation of PI and a 405 nm laser for the excitation of Hoechst 33342.

  ▲ **Note:** PI is most commonly detected in the red fluorescence channel (B3 channel on MACSQuant Analyzer 10) used for R-phycoerythrin (PE)-Cy™5 detection, it is also detectable in the yellow fluorescence channel (B2 channel on MACSQuant VYB) used for PE detection. Hoechst 33342 is detected in the blue fluorescence channel of the violet laser (V1 channel on MACSQuant Analyzer 10 and MACSQuant VYB).

2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 1 mL of buffer containing Hoechst 33342 Solution at a final concentration of 15 µg/mL.
4. Mix well and incubate for 13 minutes at room temperature.

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
5. Add the Propidium Iodide Solution at a final concentration of 1 µg/mL.
6. Incubate for additional 2 minutes at room temperature.
7. Immediately proceed with measurement using a flow cytometer, e.g., MACSQuant VYB.

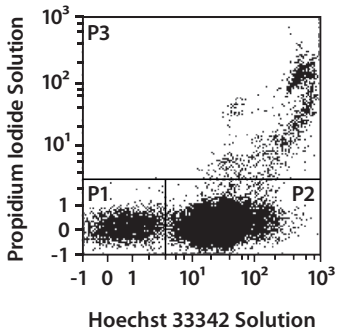
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3. Examples of immunofluorescent staining with the Live Cell Discrimination Kit

Human peripheral blood mononuclear cells (PBMCs) were stained using the Live Cell Discrimination Kit and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris was excluded from the analysis based on scatter signals.

P1 shows the debris and erythrocytes, P2 the viable cells, and P3 the PI^+ dead cells.



Gate	Events	Gated (%)
P1	2216	6.4
P2	31312	90.1
P3	1208	3.5

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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