

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human Cyclin D1: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 608030

Isotype: mouse IgG₁

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

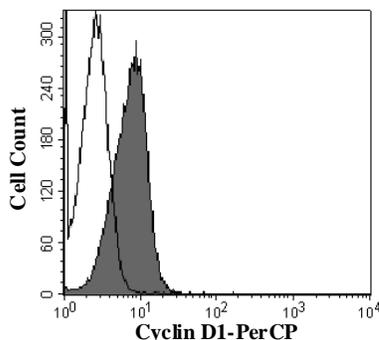
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Cyclin D1 within a population and qualitatively determine the density of intracellular Cyclin D1 by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived, recombinant human Cyclin D1 (rhCyclin D1; aa 1 - 295; Accession # NP_444284). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Intracellular expression of Cyclin D1 is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



Daudi cells were stained with PerCP-conjugated anti-human Cyclin D1 (Catalog # IC43141C, filled histogram) or isotype control (Catalog # IC002C, open histogram).

Background Information

Cyclin D1 is a 44 kDa cell cycle regulatory protein whose expression level and nuclear/cytoplasmic distribution are tightly regulated in synchrony with the cell cycle. Cyclin D1 complexes containing Cdk4 or Cdk6 induce Rb phosphorylation, a requirement for progression through the G1/S cell cycle transition. Human Cyclin D1 shares 93% amino acid sequence identity with mouse and rat Cyclin D1.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see **Reagents Not Provided**).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Resuspend 5×10^5 cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 µL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG, antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.