

5'-Bromo-2'-deoxy-uridine Labeling and Detection Kit III

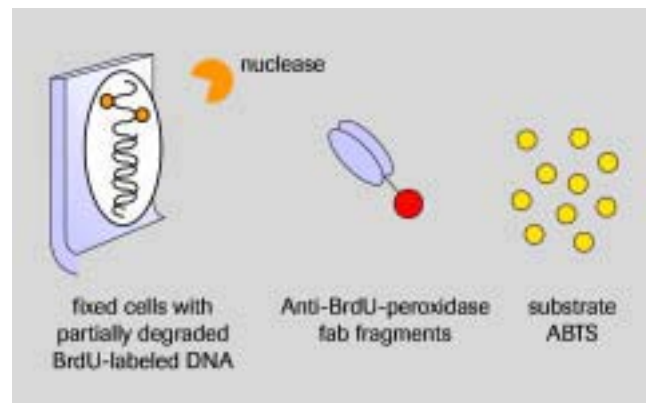
Cat. No. 11 444 611 001 1000 tests

Type	1st generation ELISA with colorimetric detection
Useful for	Quantitation of DNA synthesis during cell activation and proliferation
Sample	Adherent or suspension cell cultures
Method	Incubation of cells with BrdU, followed by partial digestion of DNA and immunodetection of incorporated BrdU label
Time	2.5–6 h (+ cell labeling)

Significance of kit: The BrdU Labeling and Detection Kit III measures cell proliferation by quantitating BrdU incorporated into the newly synthesized DNA of replicating cells. It offers a nonradioactive alternative to the [^3H]-thymidine-based cell proliferation assay.

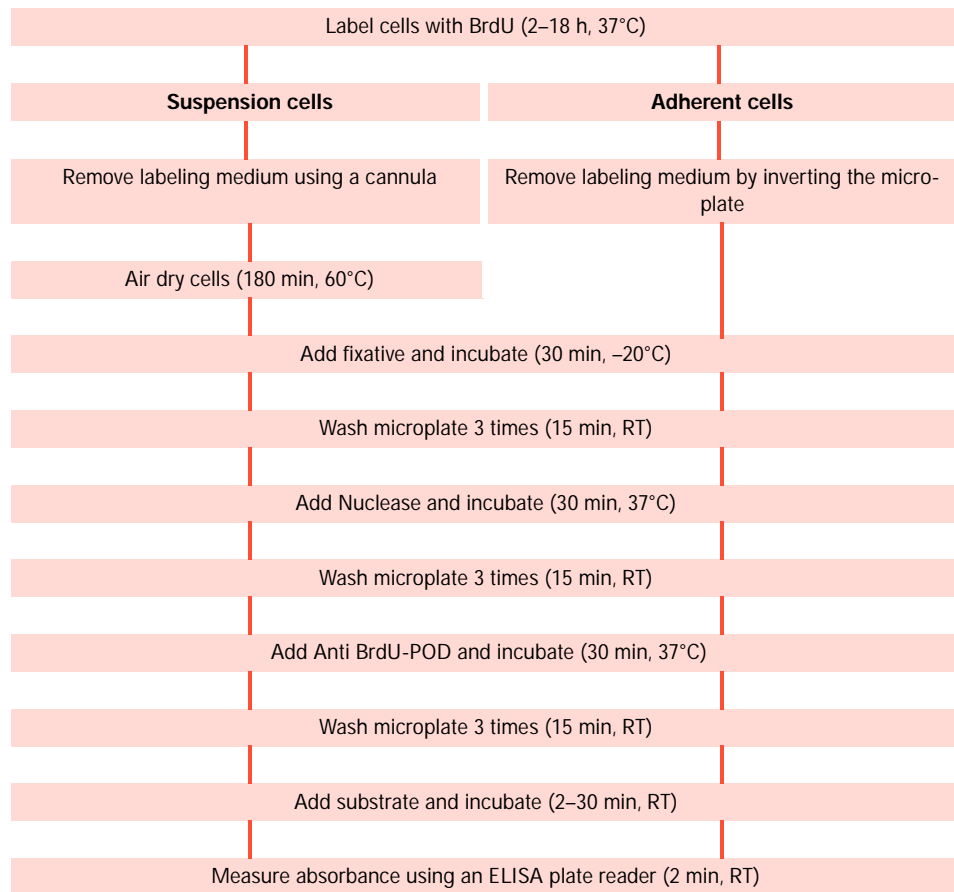
Test principle: The assay is a cellular immunoassay which uses a mouse monoclonal antibody directed against BrdU (Figure 63 and Flow Chart 16).

Note: This kit belongs to the first generation of kits used to measure DNA synthesis. The same assay procedure has been optimized and improved in the second generation Cell Proliferation ELISA, BrdU (colorimetric) kit (see Table 14).



▲ Figure 63: How the BrdU Labeling and Detection Kit III works.

B



▲ Flow Chart 16: Assay procedure, BrdU Labeling and Detection Kit III.

Sensitivity: The BrdU Labeling and Detection Kit III is almost as sensitive as the [³H]-thymidine-based cell proliferation assay. The ability to detect a minimum number of proliferating cells in a certain sample strongly depends on the amount of BrdU incorporated into the cells and thus on the labeling period. In most cases, detection requires a labeling period of 2 to 4 h.

Specificity: The antibody conjugate (Anti-BrdU-POD, Fab fragments) will bind to BrdU-labeled DNA after the DNA is denatured. The antibody is conjugated to peroxidase and specifically recognizes 5-bromo-2'-deoxyuridine; it shows no cross-reactivity with any endogenous cellular components such as thymidine or uridine.

B

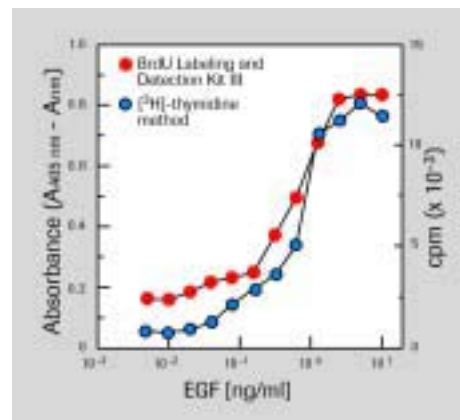
Can be used to assay:

- Adherent cells as well as cells cultured in suspension in 96-well microplates (e.g., cell lines, activated peripheral blood lymphocytes and other *in vitro* proliferating cells).

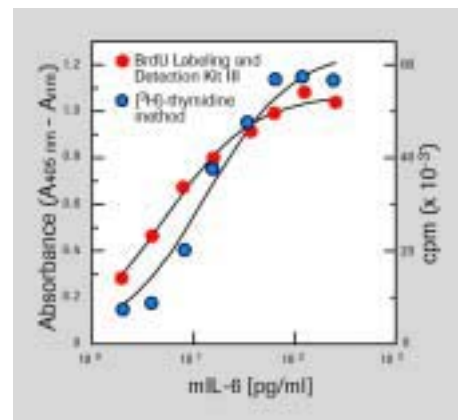
Kit contents

1. BrdU labeling reagent (1000 x), sterile
2. Anti-BrdU-POD Fab fragments
3. Incubation buffer (ready-to-use)
4. Washing buffer (10 x)
5. Nucleases
6. Substrate buffer
7. ABTS substrate tablets
8. Substrate enhancer

Typical results:



▲ Figure 64: Proliferation of AKR-2B cells (mouse fibroblast cell line) in response to recombinant human epidermal growth factor (hEGF) using the BrdU Labeling and Detection Kit III (●), or the $[^3\text{H}]$ -thymidine method (●), respectively.



▲ Figure 65: Proliferation of 7TD1 cells (mouse-mouse hybridoma) in response to recombinant mouse interleukin-6 (mIL-6) using the BrdU Labeling and Detection Kit III (●), or the $[^3\text{H}]$ -thymidine method (●), respectively.

Result: Figures 64 and 65 illustrate the equivalent sensitivity of the BrdU and $[^3\text{H}]$ -thymidine methods in measuring proliferation, as shown in hEGF and mIL-6 stimulation assays.

Other applications: For examples of how the BrdU Labeling and Detection Kit III can be used in the lab, see Appendix, pages 149–150.

Parameter	BrdU Labeling and Detection Kit III	Cell Proliferation ELISA BrdU (colorimetric) Cell Proliferation ELISA, BrdU (chemiluminescence)
Incubation steps	3	2
Washing steps	3–4	1
Working solutions	6 (4 included in the kit)	4 (all included in the kit)
Assay time	2.5–6 h	1.5–2.5 h
Incubation temperatures	–20°C: Fixation RT: Substrate reaction 37°C: Nuclease treatment 60°C: Air drying	For Cell Proliferation ELISA, BrdU (colorimetric) each step at RT
Measuring range	Absorbance: 0.1–2.5 U (factor 25)	Same as BrdU Kit III For Cell Proliferation ELISA, BrdU (chemiluminescence): rlu/s: 10^3 – 10^6 (factor 1000)
Sensitivity	Almost as sensitive as $[^3\text{H}]$ -TdR	As sensitive as $[^3\text{H}]$ -TdR

▲ Table 14: Improvements of the assay procedure using the Cell Proliferation ELISA, BrdU (colorimetric) and Cell Proliferation ELISA, BrdU (chemiluminescence) described on the following pages.

