

## Cell Proliferation ELISA, BrdU (colorimetric)

Cat. No. 11 647 229 001 1000 tests

## Cell Proliferation ELISA, BrdU (chemiluminescence)

Cat. No. 11 669 915 001 1000 tests

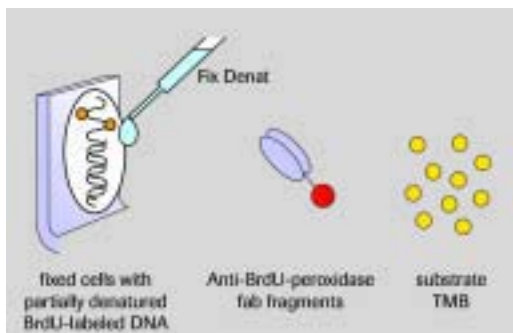
<b>Type</b>	2nd generation ELISAs with colorimetric or chemiluminescent detection
<b>Useful for</b>	Quantitation of DNA synthesis during cell activation and proliferation
<b>Samples</b>	Adherent or suspension cell cultures
<b>Method</b>	Incubation of cells with BrdU, followed by immunodetection of incorporated BrdU label
<b>Time</b>	1.5–2.5 h (+ cell labeling)

**Note:** These two kits belong to the second, improved generation of kits for measuring DNA synthesis (see Tables 14 and 15)

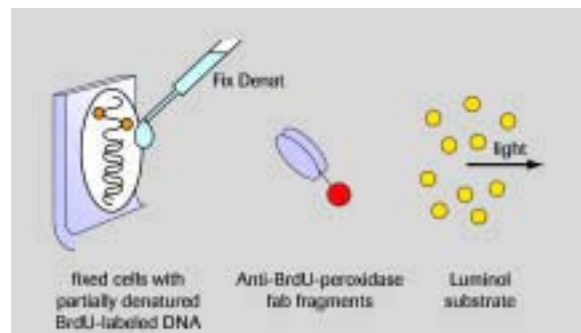
**Significance of the kits:** The two Cell Proliferation ELISAs measure cell proliferation by quantitating BrdU incorporated into the newly synthesized DNA of replicating cells. They offer a nonradioactive alternative to the [<sup>3</sup>H]-thymidine-based cell proliferation assay with comparable sensitivity.

**Test principle:** The assay is a cellular immunoassay which uses a mouse monoclonal antibody directed against BrdU. The procedure (Figures 66 and 67, Flow Chart 17) involves:

- 1 Culturing the cells in a 96-well microtiterplate and pulse-labeling them with BrdU. Only proliferating cells incorporate BrdU into their DNA.
- 2 Fixing the cells with FixDenat solution. This FixDenat solution also denatures the genomic DNA, exposing the incorporated BrdU to immunodetection.
- 3 Locating the BrdU label in the DNA with a peroxidase-conjugated anti-BrdU antibody (anti-BrdU-POD).
- 4 Quantitating the bound anti-BrdU-POD with a peroxidase substrate. TMB is used as a substrate in the Cell Proliferation, BrdU (colorimetric). Luminol/4-iodophenol is used as a substrate in the Cell Proliferation, BrdU (chemiluminescence).

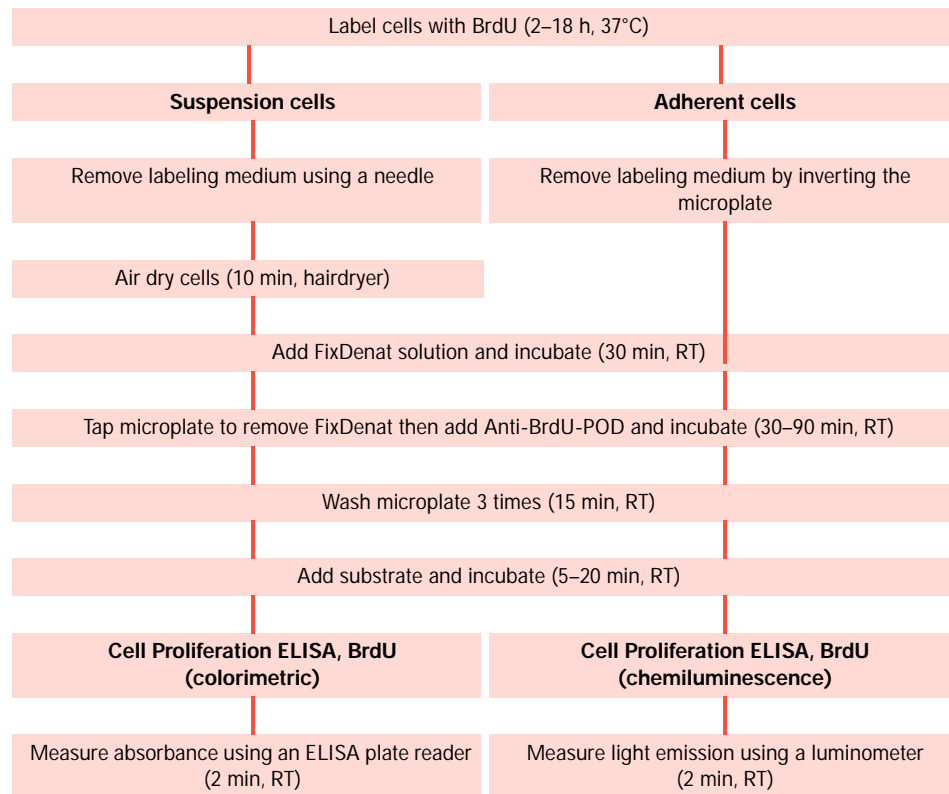


▲ Figure 66: Principle of the Cell Proliferation ELISA, BrdU (colorimetric).



▲ Figure 67: Principle of the Cell Proliferation ELISA, BrdU (chemiluminescent).

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▲ **Flow Chart 17: Assay procedures** for Cell Proliferation ELISA, BrdU (colorimetric) and Cell Proliferation ELISA, BrdU (chemiluminescence).

**Sensitivity:** The Cell Proliferation ELISA BrdU (colorimetric) and Cell Proliferation ELISA, BrdU (chemiluminescence) are as sensitive as the [<sup>3</sup>H]-thymidine-based cell proliferation assay.

**Note:** The ability to detect a minimum number of proliferating cells in a certain sample 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 24 h.

The use of a chemiluminescence substrate allows the measurement of cell proliferation over a broad range. This range is directly comparable to the measuring range of the [<sup>3</sup>H]-thymidine-based cell proliferation assay.

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

**Can be used to assay:**

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

**Kit contents**

**Cell Proliferation ELISA, BrdU (colorimetric):**

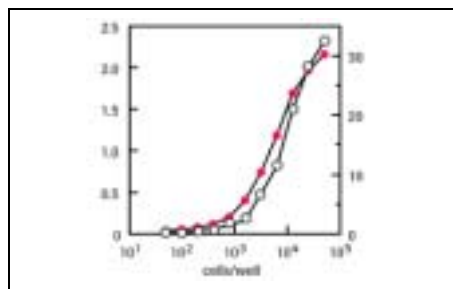
1. BrdU labeling reagent (1000 x), sterile
2. Anti-BrdU-POD Fab fragments
3. Antibody dilution solution (ready-to-use)
4. Washing buffer (10 x)
5. FixDenat (ready-to-use)
6. TMB substrate solution (ready-to-use)

**Cell Proliferation ELISA, BrdU (chemiluminescence):**

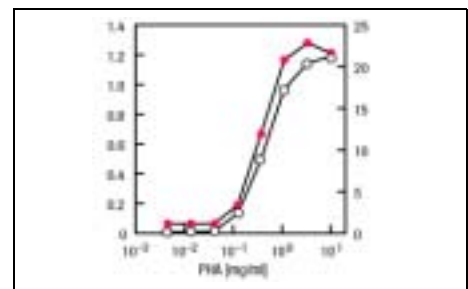
1. BrdU labeling reagent (1000 x), sterile
2. Anti-BrdU-POD Fab fragments
3. Antibody dilution solution (ready-to-use)
4. Washing buffer (10 x)
5. FixDenat (ready-to-use)
6. Substrate component A (luminol/4-iodophenol)
7. Substrate component B (peroxide)

**Note:** The FixDenat solution (included in the kits) is also available as a separate reagent (Cat. No. 11 758 764 001, 4 x 100 ml [enough for 2000 tests]). This ready-to-use solution simplifies detection of BrdU-labeled DNA in ELISA applications, since it simultaneously fixes cells and denatures DNA to expose BrdU epitopes.

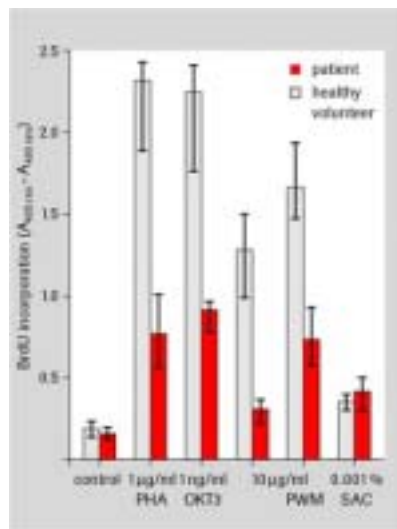
**Typical results:** see Figures 68 – 71.



▲ **Figure 68:** Comparison of the sensitivity of the Cell Proliferation ELISA, BrdU (colorimetric) and the radioactive thymidine incorporation assay for measuring proliferation in various concentrations of cells. Various concentrations of L929 cells were cultured in the wells of a microtiter plate. Duplicate cultures of each cell concentration were labeled for 4 h with either bromodeoxyuridine (BrdU) or tritiated thymidine ( $[^3\text{H}]\text{-TdR}$ ). The cells were assayed for cell proliferation with either the Cell Proliferation ELISA, BrdU (BrdU labeling, ●) or a standard filtration/liquid scintillation counting protocol ( $[^3\text{H}]\text{-TdR}$  labeling, ○). **Result:** The Cell Proliferation ELISA, BrdU (colorimetric) measures proliferation with a sensitivity comparable to the radioactive thymidine assay at all cell concentrations.

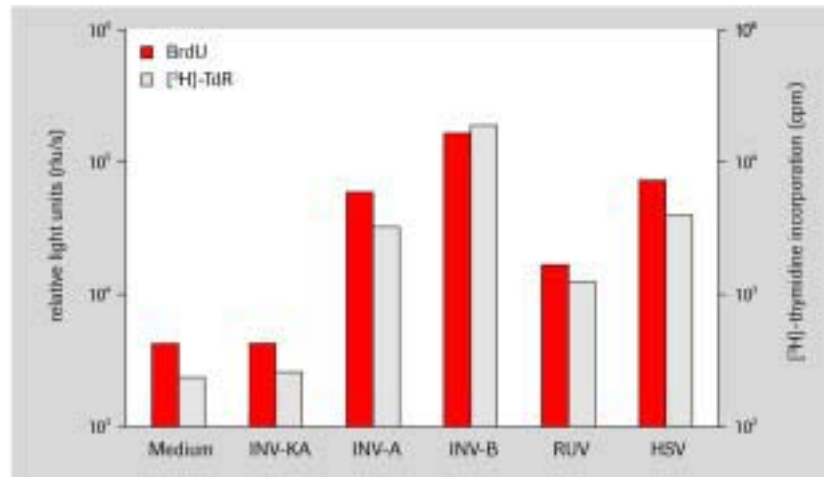


▲ **Figure 69:** Comparison of the Cell Proliferation ELISA, BrdU (colorimetric) and the radioactive thymidine incorporation assay for measuring stimulation of various concentrations of mitogen. Human peripheral blood lymphocytes were cultured in the presence of varying concentrations of phytohemagglutinin (PHA) in the wells of a microtiter plate. Duplicate cultures from each PHA concentration were labeled for 4 h with either bromodeoxyuridine (BrdU) or tritiated thymidine ( $[^3\text{H}]\text{-TdR}$ ). The cells were assayed for cell proliferation with either the Cell Proliferation ELISA, BrdU (BrdU labeling, ●) or a standard filtration/liquid scintillation counting protocol ( $[^3\text{H}]\text{-TdR}$  labeling, ○). **Result:** The Cell Proliferation ELISA, BrdU (colorimetric) is able to detect mitogen-stimulation with a sensitivity comparable to the radioactive thymidine assay.



◀ **Figure 70:** Reduced PBL proliferation of an immunosuppressed patient in response to various mitogens. Cells ( $1 \times 10^5$ /well) from a healthy volunteer (■) or an immunosuppressed individual (■) were incubated in the presence of various mitogens for 56 h. Cells were labeled with BrdU for 16 h, then cell proliferation was analyzed by Cell Proliferation ELISA, BrdU (colorimetric). The error bars indicate the maximum and minimum values of triplicate microcultures (data from T. Brüning, [1994] *Klin. Lab.* **40**, 917–927, Figure 3). Mitogens used were: PHA (phytohemagglutinin), OKT3 (anti-CD3 monoclonal antibody), Con A (concanavalin A), PWM (pokeweed mitogen), and SAC (Staphylococcus aureus Cowan I). **Result:** The BrdU ELISA clearly detected the difference in response between the healthy and immunosuppressed subjects.

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▲ **Figure 71: Measurement of the proliferation of antigen-activated PBL.** Cells ( $1 \times 10^5$ /well) were incubated in the presence of various viral antigens on culture medium alone for 5 days. After labeling with BrdU (■) or [<sup>3</sup>H]-TdR (▒) for 16 h, cell proliferation was analyzed by Cell Proliferation ELISA BrdU (chemiluminescence) (■) or LSC (▒). Antigens used were: INV-KA (influenza control antigen), INV-A (influenza virus A), INV-B, (influenza virus B), RUV (Rubella virus), and HSV (herpes simplex virus type I).

**Result:** The Cell Proliferation ELISA, BrdU (chemiluminescence) detected antigen stimulation with a sensitivity comparable to the radioactive thymidine assay.

**Other applications:** For more example of how the Cell Proliferation ELISAs can be used in the laboratory, see Appendix, pages 150–151.

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