For life science research only.
Not for use in diagnostic procedures.

Cell Proliferation ELISA, BrdU (colorimetric)

Version: 18

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Colorimetric immunoassay for the quantification of cell proliferation, based on the measurement of BrdU incorporation during DNA synthesis.
A nonradioactive alternative to the [^{3}H]-thymidine incorporation assay.

Cat. No. 11 647 229 001 1 kit
1,000 tests

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	Cell Proliferation ELISA, BrdU (colorimetric), BrdU labeling reagent, 1,000x conc.	▪ 10 mM 5-bromo-2′-deoxyuridine in PBS, pH 7.4. ▪ Filtered through 0.2 µm pore-size membrane.	1 bottle, 1 ml
2	red	Cell Proliferation ELISA, BrdU (colorimetric), FixDenat	Ready-to-use solution.	2 bottles, 100 ml each
3	blue	Cell Proliferation ELISA, BrdU (colorimetric), Anti-BrdU-POD, stabilized	▪ Lyophilized, stabilized ▪ Monoclonal antibody from mouse-mouse hybrid cells (clone BMG 6H8, Fab fragments) conjugated with peroxidase (POD).	1 bottle
4	blue	Cell Proliferation ELISA, BrdU (colorimetric), Antibody dilution solution	Ready-to-use solution.	1 bottle, 100 ml
5	green	Cell Proliferation ELISA, BrdU (colorimetric), Washing Buffer, 10x conc.	Phosphate buffered saline (PBS)	1 bottle, 100 ml
6	black	Cell Proliferation ELISA, BrdU (colorimetric), Substrate Solution	▪ Tetramethylbenzidine (TMB) ▪ Ready-to-use solution.	1 bottle, 100 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	BrdU labeling reagent, 1,000x conc.	Store at +2 to +8°C. ⚠ ***Keep protected from light.***
2	red	FixDenat	Store at +2 to +8°C.
3	blue	Anti-BrdU-POD, stabilized	
4	blue	Antibody dilution solution	
5	green	Washing Buffer, 10x conc.	
6	black	Substrate Solution	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- +37°C incubator
- Centrifuge with rotor for microplates (for suspension cells only)
- ELISA reader for microplates, with 370 nm filter without stop solution, or 450 nm filter with stop solution
  The reference wavelength should be 492 nm without stop solution, and 690 nm with stop solution.
- Microscope
- Hemocytometer
- Multichannel pipettor (10 µl and 100 µl)
- Sterile pipette tips
- Flat-bottomed 96-well microplates, tissue-culture grade

For the preparation of kit working solutions

- Double-distilled water

To stop peroxidase reaction

- 1 M H_2SO_4 (25 µl/well; = 2.5 ml/100 tests

To inhibit DNA synthesis and block proliferation

- Mitomycin C*

All other reagents required to perform 1,000 tests are included in the kit.

1.4. Application

The Cell Proliferation ELISA is designed as a precise, fast and simple colorimetric alternative to quantify cell proliferation based on the measurement of BrdU incorporation during DNA synthesis in proliferating cells. Thus, the Cell Proliferation ELISA can be used in many different *in vitro* cell systems when cell proliferation has to be determined.

- Detection and quantification of cell proliferation induced by growth factors and cytokines.
- Determination of the inhibitory or stimulatory effects of various compounds on cell proliferation in environmental and biomedical research and in the food, cosmetic, and pharmaceutical industries.
- Measurement of the immunoreactivity of lymphocytes stimulated by mitogens or antigens.
- Analysis of the chemosensitivity of tumor cells to different cytostatic drugs in medical research.

1.5. Preparation Time

Assay Time

1.5 to 3 hours depending on the Anti-BrdU-POD incubation time chosen, excluding the cell culture and labeling period.

The use of a multiwell ELISA readers allows a large number of samples to be processed simultaneously.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The Cell Proliferation ELISA can be used with adherent cells as well as suspension cells cultured in flat-bottomed 96-well microplates (tissue-culture grade), with cell concentrations and incubation periods appropriate for the respective assay in an incubator at +37°C, 5% CO_2, and 95% humidity.

Control Reactions

Blank control

Must be performed in each experimental setup. The blank provides information about the nonspecific binding of BrdU and the Anti-BrdU-POD conjugate to the microplate. The absorbance value obtained in this control should not exceed 0.1 absorbance and must be subtracted from all other values.

Background control

This is an optional control and only needs to be performed once with the respective cell system. It provides information about the nonspecific binding of the Anti-BrdU-POD conjugate to the cells in the absence of BrdU. The absorbance value obtained in this control should not exceed 0.1 absorbance. This control may significantly increase with some cell lines using high cell concentrations (more than 2 × 10^4 cells/100 µl).

Control overview

Well Contents	Blank	Background Control
Culture medium	100 µl	–
Cells	–	100 µl
BrdU	10 µl	–
Anti-BrdU-POD	100 µl	100 µl

General Considerations

Precision

To determine the intra-assay variance, various cell lines and mitogen-stimulated lymphocytes were titrated in triplicate. For all cell and mitogen concentrations tested, a variance of <10% was established for the absorbance values.

Test interference

With some cell lines, higher cell concentrations (>2 × 10^4 cells/well) may lead to increasing absorbance values in the absence of BrdU.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation	Storage and Stability	For use in...
BrdU labeling solution	▪ Dilute BrdU labeling reagent (Bottle 1) 1:100 with sterile culture medium (resulting concentration: 100 µM BrdU). ▪ For one 96-well microplate, use 1 ml BrdU labeling solution if the cells were cultured in 100 µl/well (10 µl/well) and 2 ml BrdU labeling solution if the cells were cultured in 200 µl/well (20 µl/well).	▪ Store the undiluted BrdU labeling reagent, 1,000x conc. at +2 to +8°C until the expiration date, protected from light. ▪ Store diluted BrdU labeling reagent at +2 to +8°C for several weeks. ⚠ ***Keep protected from light.*** or, For long-term storage, store in aliquots at −15 to −25°C.	Cell labeling
Anti-BrdU-POD stock solution	Dissolve Anti-BrdU-POD (Bottle 3) in 1.1 ml double-distilled water for 10 minutes and mix thoroughly.	Store at +2 to + 8°C for several months; for long-term storage, store in aliquots at −15 to −25°C.	Preparation of the Anti-BrdU-POD working solution.
Anti-BrdU-POD working solution	▪ Dilute Anti-BrdU-POD stock solution 1:100 with Antibody dilution solution (Bottle 4). ▪ For one 96-well microplate, dilute 100 µl Anti-BrdU-POD stock solution in 10 ml Antibody dilution solution (Bottle 4).	Prepare shortly before use, do not store.	Binding of the POD-labeled Anti-BrdU antibody.
Washing solution	▪ Dilute Washing Buffer concentrate (Bottle 5) 1:10 with double-distilled water. ▪ For one 96-well microplate, dilute 10 ml Washing Buffer concentrate (Bottle 5) with 90 ml double-distilled water.	Store at +2 to +8°C for several weeks.	Removal of unbound antibodies.

2.2. Protocols

Assay protocol

The general assay protocol is shown below:

1. Incubate cells in the presence of various concentrations of test substances, such as mitogens, growth factors, cytokines, and cytostatic drugs, in a 96-well microplate (tissue-culture grade, flat bottom) in a final volume of 100 µl/well in a humidified atmosphere at +37°C.
   i *The incubation period of the cell cultures depends on the particular experimental approach and on the cell type used for the assay. For most experimental setups, an incubation time of 24 to 120 hours is appropriate.*

2. Add 10 µl/well BrdU labeling solution if the cells were cultured in 100 µl/well (final concentration:10 µM BrdU) and incubate the cells for an additional 2 to 24 hours at +37°C.
   – If the cells were cultured in 200 µl/well, add 20 µl/well BrdU labeling solution.
   i *For most applications, a 2 hour labeling time is adequate. Prolongation of the incubation time will increase the amount of BrdU incorporated into cellular DNA and thus lead to increased absorbance values and sensitivity (Figures 1, 2, and 3).*

3. Remove labeling medium from adherent cells by tapping off or using suction.
   – For suspension cells, centrifuge the microplate at 300 × *g* for 10 minutes, then flick off or aspirate by pipetting.
   Dry cells using a hair dryer for approximately 15 minutes or place at +60°C for 1 hour.

4. The assay can be interrupted after the labeling process:

If you want...	Then...
to stop	after removal of the labeling medium and drying of the labeled cells, store the dry cells for up to one week at +2 to +8°C.
to go ahead	continue with Step 5.

5. Add 200 µl/well FixDenat (Bottle 2) to the cells.
   – Incubate for 30 minutes at +15 to +25°C.

6. Remove FixDenat solution thoroughly by flicking off and tapping.

7. Add 100 µl/well Anti-BrdU-POD working solution.
   – Incubate for approximately 90 minutes at +15 to +25°C.
   i *Alternatively, this incubation period can be varied between 30 to 120 minutes, depending on individual requirements, see section* **Troubleshooting**.

8. Remove antibody conjugate by flicking off and rinse wells three times with 200 to 300 µl/well Washing solution (1x PBS).

9. Remove Washing solution by tapping.

10. Add 100 µl/well Substrate Solution.
    – Incubate at +15 to +25°C until color development is sufficient for photometric detection, approximately 5 to 30 minutes.

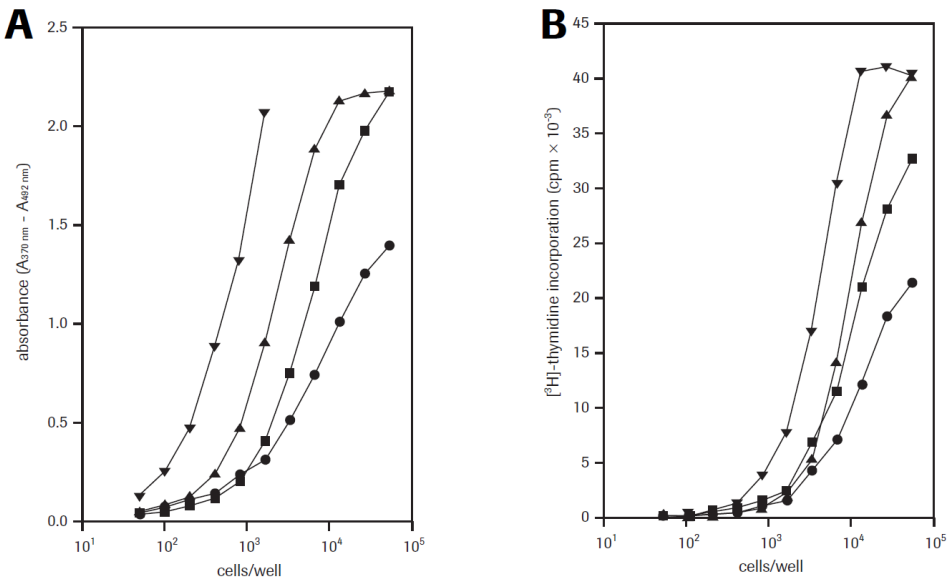

Fig. 1: Sensitivity and kinetics of the Cell Proliferation ELISA. L929 cells were titrated in flat-bottomed microplates in 100 µl/well culture medium at the concentrations indicated in the figures. After 24 hours of incubation, BrdU (A) or [3H]-thymidine (B) was added and the cells were incubated for an additional 2 hours (●), 4 hours (■), 8 hours (▲), and 24 hours (▼). BrdU incorporation was determined as described in the Assay protocol. The [3H]-thymidine incorporation assay was performed following a standard protocol.

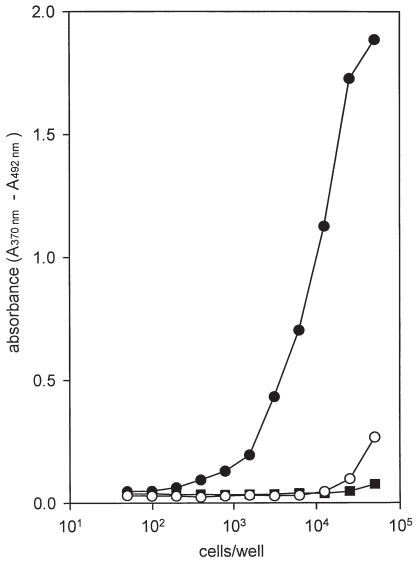

Fig. 2: Background values after treatment of cells with Mitomycin C and in the absence of BrdU. A549 cells were incubated at different concentrations in 100 µl/well culture medium with (■) or without (●, ○) Mitomycin C (5 µg/ml). After 24 hours of incubation, BrdU was added (■, ●) to the cell culture. In the respective background controls, BrdU was omitted (○). The cells were incubated for an additional 2 hours and the immunoassay was done as described in the Assay protocol

Measurement

Measurements can be done with or without stop solution.

① **Without stop solution**: measure the absorbance of the samples in an ELISA reader at 370 nm (reference wavelength approximately 492 nm).

Not stopping the substrate reaction allows repeated measurement at various times points, for example, 5, 10, and 20 minutes. Therefore, the optimal time for the substrate reaction for the respective cell system can be determined.

② **With stop solution**: add 25 µl 1 M H_2SO_4 to each well and incubate the microplate for approximately 1 minute on a shaker at 300 rpm, or mix thoroughly.
– Measure the absorbance of the samples in an ELISA reader at 450 nm (reference wavelength: 690 nm).

Measurement must be carried out within 5 minutes after adding the stop solution.

Measurement of the proliferation of mitogen-activated human peripheral blood lymphocytes (PBLs)

To study the proliferation of lymphocytes, the cells are stimulated with, for example, growth factors, cytokines, or mitogens. The increase in cell numbers can in special cases, lead to cluster formation of the lymphocytes: cells from the same progenitor stick together and form aggregates in the culture plate. This effect may disturb the antibody recognition of the ELISA system, resulting in an underestimation of the response. To avoid signal variation, carefully resuspend the cells after the BrdU-labeling period and before removing the culture medium by pipetting. This will enable the equal accessibility of each cell for the antibody recognizing the BrdU-label.

1. Titrate the mitogen Phytohemagglutinin (PHA) in the appropriate culture medium in sterile 96-well microplates by serial dilutions, for example, 1:3 to obtain a final volume of 50 µl/well.
2. For the determination of spontaneous proliferation, add 50 µl culture medium without mitogen into triplicate wells.
3. Determine the blank by adding 100 µl culture medium into triplicate wells.
4. Isolate PBLs from human peripheral blood by density gradient centrifugation, wash cells in culture medium, and dilute in culture medium to 1 × 10^6 cells/ml.
5. Add 50 µl of this cell suspension into each well except the wells required for the blank.
6. Incubate the cells in an incubator at +37°C, 5% CO_2, and 90% humidity for 48 hours.
7. Add BrdU labeling reagent and incubate for 2 to 24 hours.
8. Remove labeling medium according to the **Assay protocol, Step 3**.

Results

The results revealed from the cell proliferation ELISA strongly correlate to the data obtained by the [^{3}H]-thymidine incorporation assay. Increasing the labeling time with BrdU or [^{3}H]-thymidine up to 8 hours increases the absorbance and the cpm, respectively. A prolongation of the labeling period from 8 to 24 hours increases the absorbance values obtained in the immunoassay but reduces the cpm measured by the radioactive assay (Fig 3).

Measurement of the proliferation of allogeneic-stimulated human PBLs (mixed lymphocyte reaction, MLR)

Controls

The following controls are required for the determination of the spontaneous proliferation of responder and stimulator cells in a one- and two-way MLR.

One-Way MLR	
Stimulator control	Provides information about the BrdU incorporation of the Mitomycin C-treated stimulator cells.
Responder control I	Provides information about the spontaneous proliferation of the responder cells.
Responder control II	High values in this control indicate potential autoreactivity.
Two-Way MLR	
Syngeneic control I	Provides information about the spontaneous proliferation of the first responder cell population at the cell concentration used in the assay.
Syngeneic control II	Provides information about the spontaneous proliferation of the second responder cell population at the cell concentration used in the assay.

The following protocol describes the measurement of the proliferation of allogeneic-stimulated human PBLs (mixed lymphocyte reaction, MLR).

1. Isolate PBLs from the blood of both donors by density gradient centrifugation, wash cells in culture medium, and dilute in culture medium to 1 × 10^6 cells/ml.

2. Incubate an aliquot of allogeneic stimulator cells and syngeneic PBLs (for control) with Mitomycin C* (final concentration 25 µg/ml) in an incubator at +37°C, 5% CO_2, and 90% humidity for 30 minutes.
   ⚠ ***Keep Mitomycin C protected from light. Discard if precipitate is present.***

3. Wash Mitomycin C-treated cells at least three times in culture medium to remove free Mitomycin C.

4. Adjust cell concentration of all cell populations to 1 × 10^6 cell/ml.

5. Pipette cell suspensions into a flat-bottomed microplate according to the pipetting scheme shown below:

Sample Number (Fig 4)	Sample	Donor A	Donor B	Donor A (Mitomycin C Treated)	Donor B (Mitomycin C Treated)	Culture Medium
1	Stimulator control	–	–	–	100 µl	100 µl
2	Responder control I	100 µl	–	–	–	100 µl
3	Responder control II	100 µl	–	100 µl	–	–
4	One-way MLR	100 µl	–	–	100 µl	–
5	Syngeneic control I	200 µl	–	–	–	–
6	Syngeneic control II	–	200 µl	–	–	–
7	Two-way MLR	100 µl	100 µl	–	–	–

6 Incubate the cells in an incubator at +37°C, 5% CO_2, and 90% humidity for 5 days.

7 Add BrdU labeling reagent and incubate for 24 hours.

8 Proceed as described in the **Assay protocol, Step 3**.

Results

The results revealed from the cell proliferation ELISA strongly correlate to the data obtained by the [^{3}H]-thymidine incorporation assay (Fig. 4).

2.3. Parameters

Detection range

The immunoassay is designed to fit most of the current proliferation assays. In some cases, the absorbance values obtained may be too low or too high. See section, **Troubleshooting** on how to adapt the immunoassay to those cell systems.

Sensitivity

Depending on the individual cell type used and the incubation time applied for the assay, 0.1 to 1.0 × 10^4 cells/well are sufficient for most experimental setups with cell lines (Figures 1 and 2). Use 1 to 40 × 10^4 cells/well when working with primary lymphocytes (Figures 3 and 4).

i *The results obtained from the Cell Proliferation ELISA strongly correlate to the data obtained by the [^{3}H]-thymidine incorporation assay.*

Specificity

The antibody conjugate reacts with the thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) and with BrdU incorporated into DNA. For binding to BrdU incorporated into the DNA, the BrdU-labeled DNA has to be denatured, see section **Assay protocol**. The antibody does not cross-react with any endogenous cellular components such as thymidine, uridine, or DNA.

3. Results

Measurement of the proliferation of mitogen-activated human peripheral blood lymphocytes (PBLs)

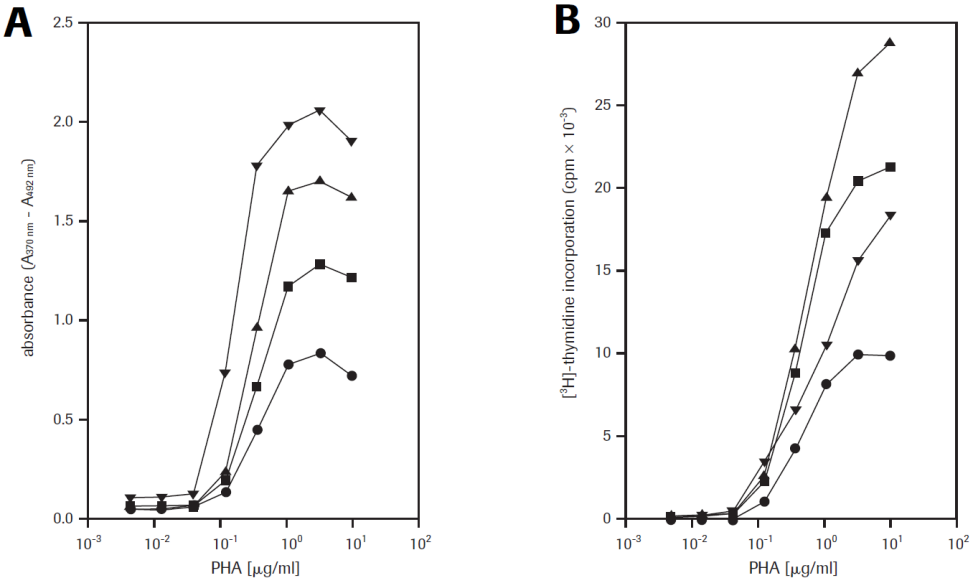

Fig. 3: PBLs were isolated and cultured in microplates for 48 hours as described in the Assay protocol. Subsequently, BrdU (A) or [3H]-thymidine (B) was added and cells were incubated for an additional 2 hours (●), 4 hours (■), 8 hours (▲), and 24 hours (▼). BrdU incorporation was determined as described in the Assay protocol. The [3H]-thymidine incorporation assay was performed following a standard protocol.

Measurement of the proliferation of allogeneic-stimulated human PBLs (mixed lymphocyte reaction, MLR)

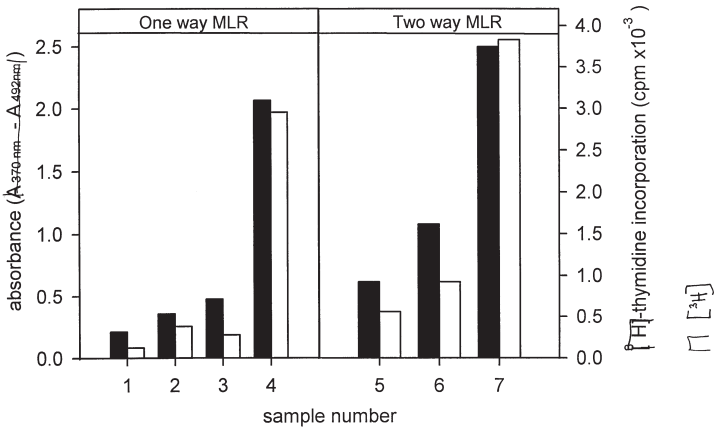

Fig. 4: Human PBLs were isolated and aliquots were treated with Mitomycin C and seeded in microplates as described in the Assay protocol. After 5 days of incubation, BrdU (closed columns) or [3H]-thymidine (open columns) was added and the cells were incubated for an additional 24 hours. Subsequently, the immunoassay was done as described in the Assay protocol. The [3H]-thymidine incorporation assay was performed following a standard protocol. The sample number in the figure can be found in Step 5 of the Measurement of the proliferation of allogeneic-stimulated human PBLs (mixed lymphocyte reaction, MLR) Protocol.

The results revealed from the Cell Proliferation ELISA strongly correlate to the data obtained by the [3H]-thymidine incorporation assay.

4. Troubleshooting

Observation	Possible cause	Recommendation
Absorbance values too low.	Low cell number.	Increase cell number or incubation time, see **Assay protocol, Step 1**.
	Labeling time too short.	Increase labeling period with BrdU to 24 hours, see **Assay protocol, Step 2**.
	Incubation time with FixDenat too short.	Increase incubation time with FixDenat to 60 minutes, see **Assay protocol, Step 5**.
	Antibody concentration too low.	Increase concentration of Anti-BrdU-POD conjugate from 2-fold to 4-fold, see **Assay protocol, Step 7**.
	Incubation time with antibody-conjugate too short.	Increase incubation time with antibody-conjugate to 2 hours and/or incubate the microplate at +37°C, see **Assay protocol, Step 7**.
	Incubation time with Substrate Solution too short.	Increase incubation time with Substrate Solution to 30 minutes, see **Assay protocol, Step 9**.
Absorbance values too high.	High cell number or incubation time.	Decrease cell number or incubation time, see **Assay protocol, Step 1**.
	Labeling time too long.	Decrease labeling period with BrdU to 2 hours, see **Assay protocol, Step 2**.
	Incubation time with FixDenat too long.	Decrease incubation time with FixDenat to 15 minutes, see **Assay protocol, Step 5**.
	Incubation time with Substrate Solution too long.	Decrease incubation time with Substrate Solution to 5 minutes, see **Assay protocol, Step 9**.
Variations of absorbance values too high.	Increase in cell numbers of lymphocytes following stimulation can lead to cluster formation of the lymphocytes: cells from the same progenitor stick together and form aggregates in the culture plate. This effect may disturb the antibody recognition of the ELISA system and thereby result in an underestimation of the response.	Avoid signal variation by carefully resuspending the cells after the BrdU-labeling period and before removing the culture medium by pipetting. This will enable the equal accessibility of each cell for the antibody recognizing the BrdU-label.
High background control.	Some cell lines show an increase in nonspecific binding of the antibody conjugate at high cell concentrations (more than 2 × 10^4 cells/well).	Reduce the cell concentration.
		After removing FixDenat in the **Assay protocol, Step 6**, add 200 µl/well Blocking Reagent* and incubate for 30 minutes at +15 to +25°C. Remove blocking solution by tapping and add Anti-BrdU-POD working solution, see **Assay protocol, Step 7**.

5. Additional Information on this Product

5.1. Test Principle

An overview of the immunoassay is shown below (Fig. 5).

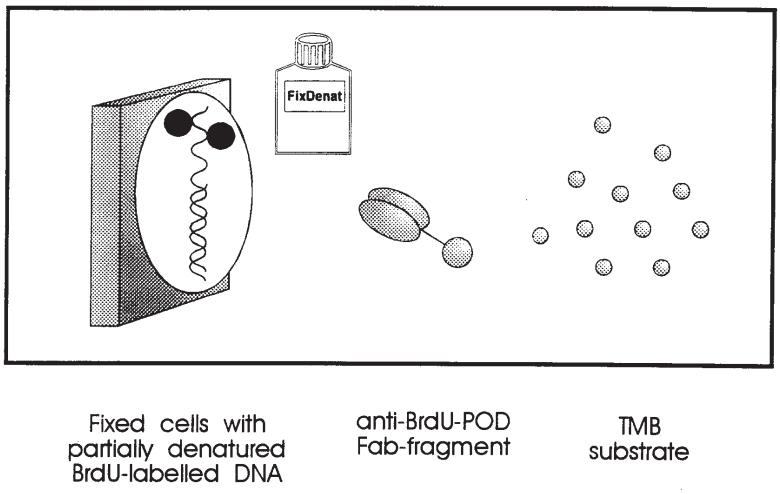

Fig. 5: Test principle

1. Cells are cultured in the presence of the respective test substances in a 96-well microplate at +37°C for 1 to 5 days, depending on the individual assay system.

2. Subsequently, BrdU is added to the cells and the cells are incubated for approximately 2 to 24 hours.
   – During this labeling period, the pyrimidine analogue BrdU is incorporated in place of thymidine into the DNA of proliferating cells.

3. After removing the culture medium, the cells are fixed and the DNA is denatured in one step by adding FixDenat.
   The denaturation of the DNA is necessary to improve the accessibility of the incorporated BrdU for detection by the antibody.

4. The Anti-BrdU-POD binds to the BrdU incorporated in newly synthesized, cellular DNA.

5. The immune complexes are detected by the subsequent substrate reaction.

6. The reaction product is quantified by measuring the absorbance at the respective wavelength using a scanning multiwell spectrophotometer (ELISA reader).
   – The developed color and thereby the absorbance values directly correlate to the amount of DNA synthesis and hereby to the number of proliferating cells in the respective microcultures.

Determination of cell proliferation

Traditionally, cell proliferation *in vitro* is determined by counting cells directly,
- by the determination of the mitotic index or,
- in the case of hematopoietic cells, by performing a clonogenic assay.

All these assays are labor-intensive and therefore not practical for evaluating large numbers of samples.

Indirect measurement of cell proliferation

Alternatively, as an indirect measure of viable cell number, the overall metabolic activity in a cell population may be determined. Tetrazolium salts such as MTT*, XTT*, or WST-1* are metabolized by NAD-dependent dehydrogenase activity to form a colored reaction product. In these assays, the amount of dye formed directly correlates to the number of viable cells. These assays are performed in a 96-well microplate and the results are easily quantified with a standard ELISA reader, allowing the processing of large sample numbers. However such assays, which measure the number of metabolically active cells, would fail when, for example, a small number of proliferating cells are masked by an overwhelming majority of non-proliferating cells (e.g., antigen-specific stimulation of lymphocytes); or when DNA synthesis is induced in an arrested cell population without any change in cell number or cell viability (e.g., short-term measurement of growth factor activity on 3T3 or AKR-2B cells).

Measurement of DNA synthesis with [^{3}H]-thymidine

Since cellular proliferation requires the replication of cellular DNA, the monitoring of DNA synthesis is another indirect parameter of cell proliferation as well as being suitable for the study of the regulation of DNA synthesis itself. DNA synthesis has been the most common measure of mitosis and cell proliferation, and [^{3}H]-thymidine has traditionally been used to label the DNA of mitotically active cells.
Disadvantages of the [^{3}H]-thymidine incorporation assay are:

- the necessity of radioisotopes,
- the inherent handling and disposal problems, and
- the requirement of specialized and expensive equipment such as a cell harvester and scintillation counter, and the hazard caused by the handling of toxic scintillation fluids.

These problems have led to the pursuit of nonradioactive replacements for this assay.

Nonradioactive measurement of DNA synthesis

An important development has been the replacement of [^{3}H]-thymidine by 5-bromo-2′-deoxyuridine (BrdU). This technique is based on the incorporation of the pyrimidine analogue BrdU instead of thymidine into the DNA of proliferating cells. After its incorporation into DNA, BrdU is detected by immunoassay. Several monoclonal antibodies which are highly specific for BrdU have been described. Originally, immunohistochemical detection of cells during the S-phase and quantification of cell proliferation has been done by microscopic or flow cytometric analysis of the cell samples. Although very informative, these techniques do not allow a high sample throughput in routine cell proliferation analysis.

How this product works

It has been shown that a precise evaluation of cell proliferation could be performed by the measurement of BrdU incorporation in newly synthesized cellular DNA. In addition, there is a good correlation between the Cell Proliferation ELISA using BrdU and the [^{3}H]-thymidine incorporation assay as shown for a variety of murine and human cell systems, including mitogen- and antigen-stimulated lymphocytes and cytokine-induced proliferation of different cell lines.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Blocking Reagent	27 g, for one liter blocking solution, *Not available in US*	11 112 589 001
Cell Proliferation Kit I (MTT)	1 kit, 2,500 tests	11 465 007 001
Mitomycin C	2 mg	10 107 409 001
Cell Proliferation Kit II (XTT)	1 kit, 2,500 tests	11 465 015 001
Cell Proliferation Reagent WST-1	8 ml, 800 tests	05 015 944 001
	25 ml, 2,500 tests	11 644 807 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany