GE Healthcare

# Amersham Cell Proliferation Biotrak ELISA System, version 2

10 x 96 wells

## Product Booklet

Code: RPN250

# Page finder

# 1. Legal



http//www.gehealthcare.com/lifesciences

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# 2. Handling

## 2.1. Safety warning and precautions

**Warning:** ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet (s) and/or safety statements for specific advice.

## 2.2. Storage

Store at 2–8°C.

## 2.3. Expiry

The expiry date is stated on the package and will be at least four weeks from the date of despatch.

# 3. Components of the assay system

The pack contains the following assay components, sufficient material for 10 x 96 wells. All components for this kit should be stored at 2–8°C.

**BrdU labelling reagent**
Bottle 1 (glass vial with red flip-up cap)
10 mM 5-bromo-2'-deoxyuridine in PBS, pH 7.4, 1000x concentrate; sterile, 1 ml. Contains bromo-deoxyuridine.

**Fixative (2 bottles)**
Bottle 2 (plastic bottle with red cap)
2 x 100 ml, ready for use. Contains ethanol.

**Blocking reagent**
Bottle 3 (plastic bottle with green cap)
Bottle contains blocking reagent supplied as a solid.

**Peroxidase-labelled anti-BrdU**
Bottle 4 (glass vial with blue cap)
Monoclonal antibody from mouse cells (Fab fragments) conjugated to peroxidase, lyophilised and stabilised.
Contains 3.5% potassium hexacyanoferrate II.

**Antibody dilution solution**
Bottle 5 (plastic bottle with blue cap)
100 ml, ready for use.

**Wash buffer (4 bottles)**
Bottle 6 (plastic bottle with green cap)
PBS, 10x concentrate, 4 x 100 ml.

**Substrate solution**
Bottle 7 (brown plastic bottle)
Bottle contains 3,3'5,5'–tetramethylbenzidine (TMB), in 15%(v/v) dimethylsulphoxide (DMSO) 100 ml, ready for use.

# 4. Critical parameters

The following points are critical:

- Allow samples and all reagents to reach room temperature before assay.
- Mix samples and all reagents prior to use.
- Avoid excessive foaming of reagents.
- Avoid handling tops of wells both before and after filling.
- It is important that all wells are washed thoroughly and uniformly.

# 5. Additional equipment and reagents required

- Pipettes or pipetting equipment with disposable tips.
- Centrifuge with rotor adaptor for microplates (for suspension cells only).
- Spectrophotometric plate reader capable of measuring at 450 nm.
- 1.0 M sulphuric acid. 1 M sulphuric acid may be prepared by carefully adding 560 µl concentrated sulphuric acid to 8 ml of ice-cold distilled water. Carefully mix and make up to 10 ml with water.
- Microscope.
- Haemocytometer.
- Flat-bottomed 96-well microplate, tissue culture grade.
- Tissue culture incubator.
- Tissue culture medium.
- Distilled or deionised water.

# 6. Description

The Biotrak™ cell proliferation ELISA system, version 2 from GE Healthcare has been specifically designed for research purposes. Traditionally, the measurement of cell proliferation has involved the use of [$^3$H]-thymidine to allow monitoring of DNA synthesis. The cell proliferation ELISA is designed as a precise, fast and simple colorimetric alternative to quantitate cell proliferation based on the measurement of 5-bromo-2′-deoxyuridine (BrdU) incorporation during DNA synthesis in proliferating cells. This technique is based on the incorporation of the pyrimidine analogue BrdU instead of thymidine into the DNA of proliferating cells.

The principle of the assay involves culturing cells in the presence of the respective test substances in a 96-well microplate at 37°C for 1 to 5 days. BrdU is added to the cells and these are reincubated (usually 2–24 hours). During this labelling period, the pyrimidine analogue BrdU is incorporated in place of thymidine into the DNA of proliferating cells. After removing the culture medium, the cells are fixed and the DNA denatured by addition of fixative (the denaturation of the DNA is necessary to improve the accessibility of the incorporated BrdU for detection by the antibody). The peroxidase-labelled anti-BrdU binds to the BrdU incorporated in newly synthesised, cellular DNA. The immune complexes are detected by the subsequent substrate reaction, and the resultant colour read at 450 nm in a microplate spectrophotometer. The absorbance values correlate directly to the amount of DNA synthesis and thereby to the number of proliferating cells in culture.

Each pack contains sufficient material for 10 x 96 wells. The Biotrak cell proliferation assay offers a number of key advantages:

**Non-radioactive**

- No radioactive isotopes are used.

**Accurate**

- High level of correlation with the number of proliferating cells.
- Low mean variation.

**Sensitive**

- As least as sensitive as [$^{3}$H]-thymidine incorporation.

**Fast**

- The immunoassay can be performed in approximately 3 hours.
- The use of a multiwell ELISA reader allows a large number of samples to be processed simultaneously.

**Convenience**

- All labelling and detection components supplied with extensive protocols.
- No disposal of radioactive isotopes.
- Reagents are provided in stable, optimised form.
- Directly labelled anti-BrdU.
- No transfer of cells; the assay is carried out in single microplate wells.
- 1 wash and 4 incubation steps only.
- The entire immunoassay is carried out at room temperature.
- Mild fixation and DNA denaturation preserves cellular morphology and thus allows optical control of the cells during the assay.
- Ready to use substrate.

**Function tested**

- Each batch is function tested on proliferating cells and results compared with a master batch.

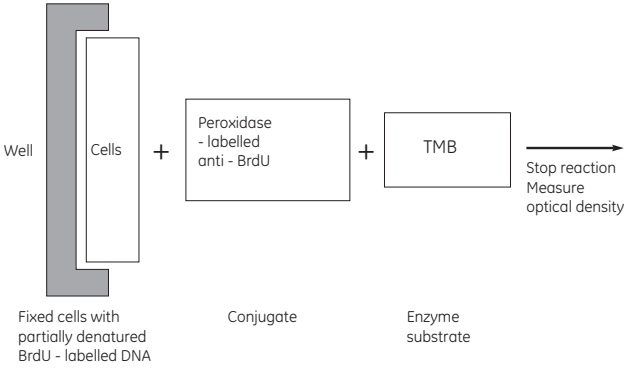


**Figure 1.** Assay principle

# 7. System protocol

## 7.1. Reagent preparation

**Storage**

Before reconstitution, store the reagents at 2–8°C. The expiry date is stated on the package and will normally be at least four weeks from the date of despatch.

**Note:** All reagents must be allowed to fully equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation.

**Solution A. BrdU labelling solution (stock concentrate contains 10 mM BrdU)**

1. Dilute the BrdU labelling reagent (bottle 1, glass vial with red flip-up cap) 1:1000 with sterile culture media (final concentration: 10 µM BrdU). For one 96-well microplate containing 100 µl/well culture medium, 10 ml BrdU labelling solution is required.
2. Dilute 10 ml of BrdU labelling reagent with 10 ml sterile culture media to give 1:1000 dilution.
3. If the cells are cultured in 200 µl/well, add 20 µl/well BrdU labelling solution to 20 ml sterile culture media. An alternative method for preparing the BrdU labelling solution is described in the assay protocol section.

   The undiluted BrdU labelling reagent (1000 x) should be stored protected from light and is stable at 2–8°C for several months. The diluted BrdU labelling solution is stable for several weeks and should be stored protected from light at 2–8°C. For long term storage it is recommended to store the BrdU labelling solution in aliquots at -15°C to -30°C.

**Solution B. Blocking reagent**

1. Dissolve the contents of the bottle (bottle 3) in 100 ml redistilled

water at room temperature for 30 minutes to obtain a relatively clear solution.

This concentrate is stable when stored in aliquots at -15°C to -30°C.

2. For use dilute 1 aliquot at a ratio of 1:10 with redistilled water to yield a working solution containing 1%(w/v) protein in 50 mM Tris-HCl; 150 mM NaCl, pH7.4.

Avoid prolonged storage of solutions at 2–8°C or at room temperature since solutions of the blocking reagent are excellent media for the growth of micro-organisms.

**Solution C. Peroxidase-labelled anti-BrdU, stock solution**

1. Add 1.1 ml redistilled water to the peroxidase-labelled anti-BrdU (bottle 4, glass vial with blue cap) and thoroughly mix for 10 minutes until the contents are completely dissolved.

This stock solution is stable at 2–8°C for several months. For long-term storage, it is recommended to store the solution in aliquots at -15°C to -30°C.

**Solution D. Peroxidase-labelled anti-BrdU, working solution**

1. Dilute the peroxidase-labelled anti-BrdU stock solution (solution C) 1:100 with antibody dilution solution (bottle 5, blue cap).
2. For one 96-well microplate, add 100 µl peroxidase-labelled anti-BrdU stock solution (solution C) to 9.9 ml antibody dilution solution (bottle 5, blue cap).

The peroxidase-labelled anti-BrdU working solution should be prepared shortly before use and should not be stored.

**Solution E. Wash buffer**

1. Dilute the wash buffer concentrate (bottle 6, green cap) 1:10 with redistilled water. For one 96-well microplate, add 10 ml wash buffer concentrate (bottle 6, green cap) to 90 ml redistilled water.

The wash buffer is stable at 2–8°C for several weeks.

## 7.2. Assay protocol

**Note:** Equilibrate all kit reagents to room temperature and mix before use. This is particularly important with the enzyme substrate, TMB.

**Controls**

**Assay blank**

This should be included in each experiment. The assay blank provides information on non-specific binding of BrdU and peroxidase-labelled anti-BrdU to the microplate. The absorbance value obtained from this control should not exceed 0.1 absorbance units and should be subtracted from all other values.

**Non-specific binding control**

The non-specific binding control provides information on the binding of the peroxidase-labelled anti-BrdU to the cells in the absence of BrdU. The absorbance value from this control should not exceed 0.1 absorbance units. The absorbance values for this control assay may increase significantly with some cell lines, with high cell concentrations (greater than $2 \times 10^4$ cells/well).

**Table 1.** Summary of controls

| Well contents | Assay blank | Non-specific binding control |
|---|---|---|
| Culture medium | 100 µl | – |
| Cells | – | 100 µl |
| BrdU | 10 µl | – |
| Peroxidase-labelled anti-BrdU | 100 µl | 100 µl |
| Substrate | 100 µl | 100 µl |
| Stop solution | 25 µl | 25 µl |

**Cell culture**

1. Culture cells together with various dilutions of test substance (eg. mitogens, growth factors, cytokines, 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.
2. The incubation period for the cell cultures depends on the particular experimental approach and on the cell type used for the assay. For most experiments an incubation time of 24 to 120 hours is appropriate. Cells should be passaged and cultured under the conditions routinely employed for the cell line being used for the assay. Where possible, avoid the use of media containing thymidine - NCTC 135, MEM-Alpha, F10, F12 etc. Media may be used with or without serum supplementation, as appropriate to the experiment. Use $10^3$-$10^5$ cells/well for assay, depending on the duration of the experiment.

**Labelling the cells with BrdU**

1. Resuspend cells in BrdU labelling solution (solution A, see page 11) containing 10 µM BrdU and dispense into a 96-well cluster plate.
2. Alternatively dilute the BrdU labelling reagent (bottle 1) 1:100 with sterile culture media to give a final concentration of 100 µM BrdU and add 10 µl/well BrdU labelling solution directly to cultured cells, if cells are cultured in 100 µl/well volumes (final concentration: 10 µM BrdU). If cells are cultured at 200 µl/well, add 20 µl/well BrdU labelling solution.
3. Reincubate cell cultures for an additional 2 to 24 hours at 37°C. For most applications, a 2 hour labelling time is adequate. Extension of the incubation time will increase the amount of BrdU incorporated into cellular DNA and thus will increase absorbance values and sensitivity.

**Removal of labelling medium**

**For adherent cells**

Remove labelling medium by tapping or suction

**For suspension cells**

1. Centrifuge the microtitre plate at 300 xg for 10 minutes and remove the labelling medium by tapping or suction.
2. Dry cells using a hairdryer for about 15 minutes or at 60°C for 1 hour.

**Cell fixation and DNA denaturation**

1. Add 200 µl/well fixative (bottle 2) to the cells and controls, and incubate for 30 minutes at room temperature.

**Blocking**

1. Remove fixative solution by tapping.
2. Add 200 µl/well blocking buffer (solution B) to cells and controls.
3. Incubate for 30 minutes at room temperature.

**Incubation with peroxidase-labelled anti-BrdU**

1. Remove the blocking buffer by tapping.
2. Add 100 µl/well peroxidase-labelled anti-BrdU working solution (solution D, see page 12).
3. Incubate for approximately 90 minutes at room temperature. This incubation period can be varied between 30–120 minutes depending on individual conditions.

**Washing**

1. Remove antibody conjugate by tapping.
2. Rinse wells three times with 200 µl-300 µl/well washing solution (solution E, see page 12).

   If a plate washing machine is used, care should be taken to ensure that wash nozzles do not scrape cells from the bottom of

the wells. The washing step is important for good performance and must be done thoroughly. Blot carefully.

**Substrate reaction**

1. Immediately dispense 100 µl of room temperature equilibrated TMB substrate into all wells.
2. Cover the plate and mix at room temperature until colour development is sufficient for optical density measurement (5–30 minutes). Incubation times for colour development will vary according to experimental conditions. For example, in a dose-response experiment the colour reaction should be stopped when a clear gradation of colour is visible between wells. Over development may lead to saturation of colour in rapidly developing wells and raised blanks.
3. When the required colour intensity is achieved, stop the reaction by pipetting 25 µl of 1 M sulphuric acid into each well.
4. Determine the optical density in a plate reader at 450 nm within 5 minutes.

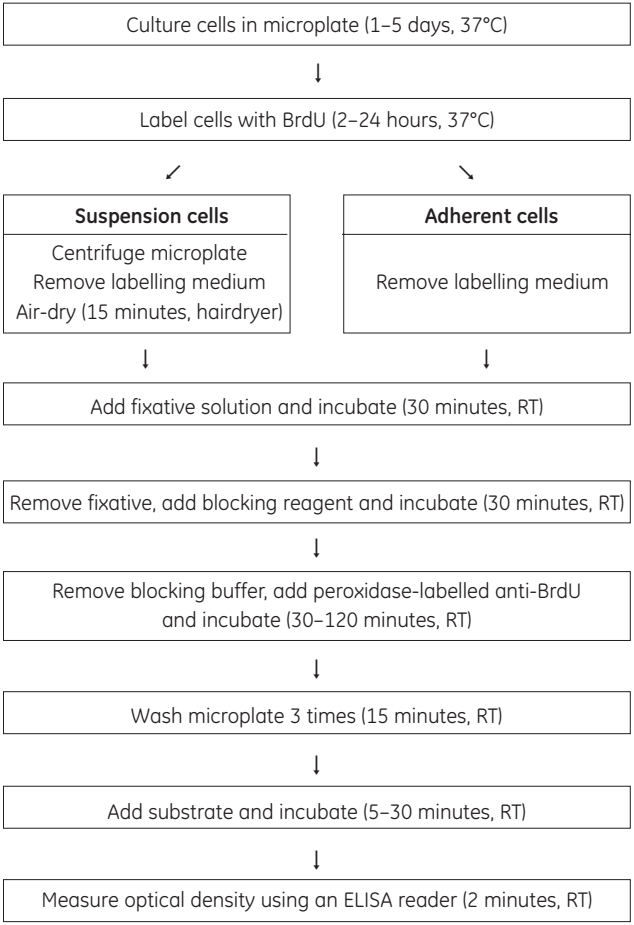


**Figure 2.** Flow chart of assay procedure

# 8. Additional information

## 8.1. Specificity

The antibody conjugate binds to thymidine analogue 5-bromo-2'-deoxyuridine (BrdU) and with BrdU incorporated into DNA. For binding to BrdU incorporated into DNA, the BrdU labelled DNA has to be denatured. The antibody does not cross-react with any endogenous cellular components such as thymidine, uridine or DNA.

## 8.2. Sensitivity

Depending on the individual cell type and the incubation time used for the assay, $10^3$-$10^4$ cells/well are sufficient for most experiments. $10^4$- 4x$10^5$ cells/well should be used when dealing with primary lymphocytes.

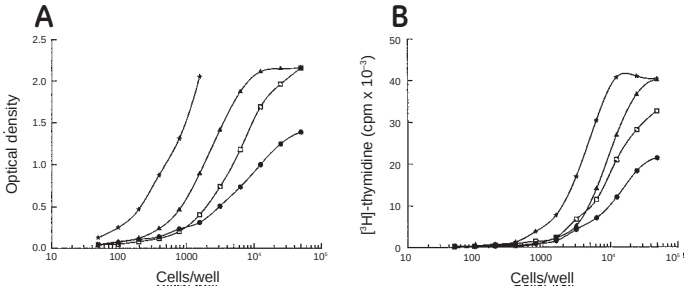


**Figure 3:** Sensitivity and kinetics of the cell proliferation ELISA

L929 cells were cultured for 24 hours in microplates. After 24 hours incubation, BrdU (A) or [$^3$H]thymidine (B) were added and the cells reincubated for an additional 2 hours (●), 4 hours (□), 8 hours (▲) or 24 hours (*). BrdU incorporation was determined as described in the assay procedures section. The [$^3$H]thymidine incorporation assay was carried out using a standard protocol.

**Note:** The results revealed from the cell proliferation ELISA strongly correlated with data obtained using the [$^3$H]thymidine incorporation assay. Increasing the labelling time with BrdU or [$^3$H]thymidine increased the absorbance and the cpm respectively. In this assay system 24 hours labelling with BrdU resulted in increased sensitivity compared with the [$^3$H]thymidine incorporation assay at low cell concentrations. At higher cell concentrations the prolonged labelling time resulted in optical densities beyond the measuring range of the ELISA reader.

## 8.3. Precision

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

## 8.4. Test interference

With some cell lines, high cell concentrations (greater than $2 \times 10^4$ cells/well) may lead to increasing absorbance values in the absence of BrdU.

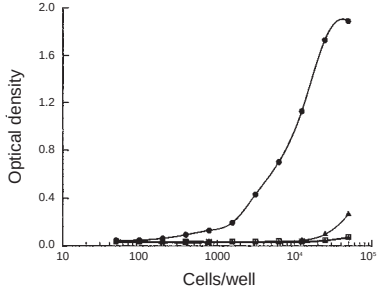


**Figure 4:** Background values after treatment of cells with mitomycin C in the absence of BrdU.

A549 cells were incubated with (*, ☐) or without (●, ■) mitomycin (5 mg/ml). After incubating for 24 hours, BrdU was added (*, ●). In the respective background control, BrdU was omitted (▲, ☐). The cells were reincubated for an additional 2 hours and BrdU incorporation was measured as detailed in the assay procedures section.

## 8.5. Sample material

Culture cells (adherent and in suspension) in flat-bottomed 96-well microplates (tissue culture grade), with cell concentrations and incubations appropriate for the respective assay. Set incubator at 37°C, with 5% $CO^2$ and 95% humidity.

## 8.6. Assay time

3-4 hours, depending on the peroxidase-labelled anti-BrdU incubation time, excluding the cell culture and labelling period.

## 8.7. Examples

**Example 1.** Measurement of the proliferation of mitogen- activated, human peripheral blood lymphocytes (PBLs) (12).

**Assay procedure**

1. Titrate mitogen (for example, phytohaemoagglutinin, PHA) in the appropriate culture medium in sterile 96-well microplates by serial dilutions (eg 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 give 1 x $10^6$ cells/ml.

5. Add 50µl of this cell suspension into each well except the wells required for the blank.

6. Incubate the cells in an incubator (37°C, 5% $CO^2$, 90% humidity) for 48 hours.

7. Add BrdU labelling reagent and reincubate for 2 to 24 hours.

8. Proceed as described in the assay protocol section (see page 17).

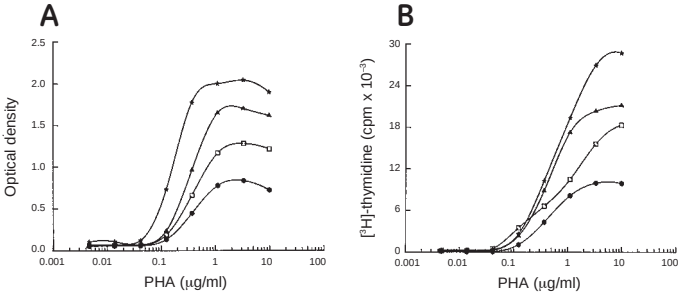


**Figure 5.** Measurement of the proliferation of mitogen-activated, human peripheral blood lymphocytes (PBLs).

PBLs were isolated and cultured for 48 hours. Subsequently BrdU (A) or [$^3$H]thymidine (B) were added and cells reincubated for an additional 2 hours (●), 4 hours (□), 8 hours (▲) or 24 hours (*). BrdU incorporation was determined as described in the assay procedures section. The [$^3$H]thymidine incorporation assay was carried out following standard methods.

**Note:** The results from the cell proliferation ELISA strongly correlate with those from the [$^3$H]thymidine incorporation assay.

**Example 2.** 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 mixed lymphocyte reaction:

**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 oncentration 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.

**Table 2.** Assay summary for mixed lymphocyte reaction

| Sample Number | Sample | Donor A | Donor B | Donor A | Donor B | 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 | – | – | – |

**Assay procedure**

**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 x $10^6$ 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 (37°C, 5% $CO_2$, 90% humidity) for 30 minutes.

**Note:** Protect mitomycin 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 concentrations to a population of 1 x $10^6$ cells/ml.

**5.** Pipette cell suspensions in a flat-bottomed microplate, according to the scheme shown in table 2.

**6.** Incubate the cells in an incubator (37°C, 5% $CO_2$, 90% humidity) for 5 days.

**7.** Add BrdU labelling reagent and reincubate for 24 hours.

**8.** Proceed as described in the assay protocol section (see page 17).

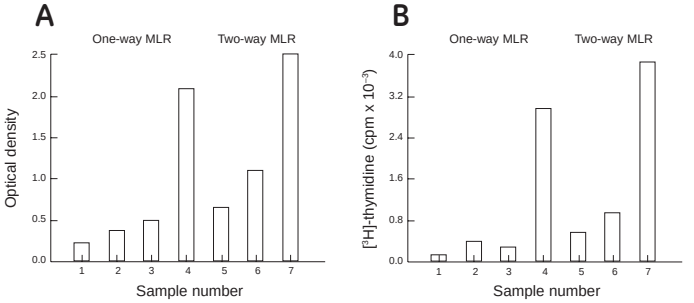


**Figure 6.** Measurement of the proliferation of allogeneic-stimulated human PBLs (mixed lymphocyte reaction, MLR)

Human PBLs were isolated, treated with mitomycin C and seeded into microplates. After 5 days of incubation, BrdU (A) or [$^3$H]thymidine (B) were added and the cells reincubated for an additional 24 hours. BrdU incorporation was measured as detailed in the assay procedures section. The [$^3$H]thymidine assay was carried out following standard methods.

**Note:** The results from the cell proliferation ELISA correlate strongly with those from the [$^3$H]thymidine incorporation assay.

# 9. Troubleshooting guide

Since the various cell culture systems greatly differ in cell number, proliferating activity of the cells and incubation periods, most problems will occur because of too high or too low absorbance values. Assay conditions may be changed in order to achieve the appropriate optical density measurement (see table 3).

| Problem | Solution |
|---|---|
| **1. Too low an absorbance** | **1.** Increase cell number or incubation time<br>**2.** Increase labelling period with BrdU to 24 hours<br>**3.** Increase incubation time with fixative to 60 minutes<br>**4.** Increase concentration of peroxidase-labelled anti-BrdU conjugate 2-fold to 4-fold<br>**5.** Increase incubation time with antibody-conjugate to 2 hours and/or incubate the microplate at 37°C<br>**6.** Increase incubation time with substrate solution to 30 minutes |
| **2. Too high an absorbance value** | **1.** Decrease cell number or incubation time<br>**2.** Decrease labelling period with BrdU to 2 hours<br>**3.** Decrease incubation time with fixative to 15 minutes |

| Problem | Solution |
|---|---|
| **2. Too high an absorbance value** *continued* | **4.** Decrease incubation time with substrate solution to 5 minutes |
| **3. High background control** | **1.** Some cell lines show an increase in non-specific binding of the antibody conjugate at high cell concentrations (more than $2x10^4$ cells/well). Reduction of the cell concentration will overcome this problem. |

# 10. Background

Traditionally, cell proliferation *in vitro* is determined by counting cells directly, by the determination of the mitotic index or, in the case of haematopoietic cells, by performing a clonogenic assay. All these assays are labour-intensive and are not practical for evaluating large numbers of samples. Alternatively, as an indirect measure of viable cell number, the overall metabolic activity in a cell population may be determined. Tetrazolium salts like MTT, XTT or WST-1 are metabolised by NAD-dependent dehydrogenase activity to form a coloured reaction product. In these assays the amount of dye formed directly correlates to the number of viable cells. These assays are performed in 96-well microplates 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 (eg 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).

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: (1) handling and disposal problems of radioisotopes; (2) the requirement of specialised and expensive equipment like a cell harvester and scintillation counter, and (3) the hazards associated with the handling

of hazardous scintillation fluids. These disadvantages have led to the development of non-radioactive replacements for this assay.

An important improvement 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 (1–6). Original techniques involved immunohistochemical detection of cells during the S-growth phase and, quantification of cell proliferation by microscopic or flow cytometric analyses. Although very informative, these techniques do not allow a high sample throughput in routine cell proliferation studies. In 1985, Porstmann *et al*, first described an enzyme immunoassay for the assessment of cell proliferation by quantification of BrdU incorporation into DNA (7). This method has been varied and optimised by several laboratories (8–11).

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

**Examples:**

- Detection and quantification of cell proliferation induced by growth factors and cytokines
- Determination of inhibitory or stimulatory effects of various compounds on cell proliferation in environmental and biomedical research and in the food, cosmetic and pharmaceutical industries
- Determination of the immunoreactivity of lymphocytes, stimulated by mitogens or antigens

- Determination of the chemosensitivity of tumour cells to different cytostatic drugs in medical research

It has been shown that a precise evaluation of cell proliferation can be carried out with the measurement of BrdU incorporation in newly synthesised 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.

# 11. References


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# 12. Related products

| | |
|---|---|
| h-sICAM-1 ELISA | RPN 247 |
| h-sELAM-1 ELISA | RPN 248 |
| h-sVCAM-1 ELISA | RPN 249 |
| Mouse sICAM-1 ELISA | RPN 2721 |

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