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

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

Version: 19

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96-well microplate cell ELISA for the detection of 5-bromo-2′-deoxy-uridine (BrdU) incorporated into cellular DNA.
Nonradioactive alternative for [^{3}H]-thymidine-based DNA synthesis and cell proliferation assays.

Cat. No. 11 444 611 001 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	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, BrdU labeling reagent, 1,000x conc.	▪ 10 mM 5-bromo-2′-deoxy-uridine in PBS, pH 7.4. ▪ Filtered through 0.2 µm pore-size membrane. ▪ For labeling of DNA.	1 bottle, 1 ml
2	colorless	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Washing Buffer, 10x conc.	▪ Phosphate buffered saline (PBS) ▪ For wash steps.	1 bottle, 125 ml
3	red	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Incubation buffer	▪ 66 mM Tris buffer, 0.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol. ▪ For preparation of the BrdU Working solution.	1 bottle, 125 ml
4	blue	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Nucleases	Stabilized, lyophilized	1 bottle
5	yellow	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Anti-BrdU-POD, Fab fragments	▪ Monoclonal antibody, Fab fragments from mouse conjugated with peroxidase, ▪ Stabilized, lyophilized ▪ For the binding of the BrdU incorporated into the DNA.	1 bottle, 25 U
6	green	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Substrate buffer	▪ ABTS substrate buffer (sodium perborate and citric acid/phosphate buffer).	1 bottle, 125 ml
7	green	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, ABTS substrate	Powder for 125 ml ABTS substrate buffer.	1 bottle
8	green	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit III, Substrate enhancer	Substrate enhancer	1 bottle, 125 mg

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	Store at +2 to +8°C.
2	colorless	Washing Buffer concentrate, 10x	
3	red	Incubation buffer	
4	blue	Nucleases	
5	yellow	Anti-BrdU-POD, Fab fragments	
6	green	Substrate buffer	
7	green	ABTS substrate	
8	green	Substrate enhancer	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- +37°C, CO_2 incubator
- Humidified chamber
- Water bath
- Centrifuge with rotor for microplates (for suspension cells only)
- Flat-bottomed, 96-well microplates, tissue-culture grade
- ELISA reader for microplates, with 405 nm filter
  The reference wavelength should be approximately 490 nm.
- Multichannel pipettes
- Sterile pipette tips
- Cannula

For preparation of kit working solutions

- Double-distilled water
- PBS* filtered through 0.2 µm pore-size membrane or culture medium
- 50% glycerol
- BSA*

For fixing cells

- 70% ethanol in HCl (final concentration 0.5 M)
  For one 96-well microplate, dilute 14 ml 100% ethanol with 4.66 ml double-distilled water and add 1.34 ml 25% HCl. Before use, precool and store fixative at −15 to −25°C.

For washing steps

- Washing medium: PBS or culture medium containing 10% serum, such as FCS (fetal calf serum)

For measurement of the proliferation of adherent cells AKR-2B

- Culture medium, such as McCoy 5 A containing 7.5% FCS (v/v) and 2 mM L-glutamine
  If an antibiotic is to be used, further supplement media with Penicillin/Streptomycin or gentamicin.*
- Synchronization medium, such as MCDB-402
- EGF, human, recombinant*
- Insulin, human, recombinant*
- BSA (for cell culture)*

For measurement of the proliferation of suspension cells 7TD1

- Culture medium, such as DMEM containing 10% heat inactivated FCS, 2 mM L-glutamine, 0.55 mM L-arginine, 0.24 mM L-Asparagine monohydrate, 50 µM 2-mercaptoethanol, 1x HT Media supplement containing 0.1 mM hypoxanthine and 16 µM thymidine.
  If an antibiotic is to be used, further supplement media with Penicillin/Streptomycin or gentamicin.*
- Interleukin-6, human, recombinant (200,000 U/ml; 2 µg/ml)*

1.4. Application

The kit is designed for the quantitative determination of BrdU incorporated into cellular DNA using a 96-well microplate cell ELISA format.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The Labeling and Detection Kit III can be used with adherent and suspension cells cultured in 96-well microplates.

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 Number	Solution	Preparation	Storage and Stability	For use in...
1	BrdU labeling solution	▪ Dilute BrdU labeling reagent (Bottle 1) 1:90 with PBS filtered through 0.2 µm pore-size membrane or culture medium (final concentration: 111 µM BrdU). ▪ For one 96-well microplate containing 100 µl medium per well, dilute 12 µl BrdU labeling reagent (Bottle 1) with 1.068 ml filtered PBS.	⚠ ***Prepare shortly before use.*** Store the undiluted BrdU labeling reagent, 1,000x conc. at +2 to +8°C for 6 months. For long-term storage, store in aliquots at −15 to −25°C.	Cell labeling.
2	Washing buffer	▪ Dilute Washing Buffer, 10x conc. (Bottle 2) 1:10 with double-distilled water. ▪ For one 96-well microplate, dilute 9 ml Washing Buffer, 10x conc. (Bottle 2) with 81 ml double-distilled water.	Store at +2 to +8°C for 3 months. ⚠ ***If precipitates are visible, incubate the bottle for 10 minutes at +37°C in a water bath before preparing Solution II.***	▪ Preparation of Anti-BrdU-POD, working solution, see Solution 5a. ▪ Washing cells after incubation with Anti-BrdU-POD. ⓘ *For all other washing steps, use PBS or culture medium containing 10% serum, such as FCS to obtain reliable results.*

3	Incubation buffer	▪ Ready-to-use solution.	Store at +2 to +8°C.	Dilution of nucleases.
4	Nucleases, stock solution	▪ Reconstitute the Nucleases, stock solution (Bottle 4) in 1.3 ml double-distilled water containing 50% glycerol (w/v).	Store at −15 to −25°C for 6 months.	Preparation of Nucleases, working solution.
4a	Nucleases, working solution	▪ Dilute Nucleases, stock solution, 1:100 with Incubation buffer (Bottle 3). ▪ For one 96-well microplate, dilute 100 µl Nucleases, stock solution (Bottle 4), with 9.9 ml Incubation buffer (Bottle 3).	⚠ ***Prepare shortly before use.***	▪ Partially digest the DNA. ▪ Improve accessibility of BrdU for detection by antibodies.
5	Anti-BrdU-POD, Fab fragments, stock solution	▪ Dissolve Anti-BrdU-POD, Fab fragments (Bottle 5) in 1.25 ml double-distilled water (final concentration: 20 U/ml).	Store at +2 to +8°C for 6 months. For long-term storage, store in aliquots at −15 to −25°C.	Preparation of Anti-BrdU-POD, Fab fragments, working solution.
5a	Anti-BrdU-POD, Fab fragments, working solution	▪ Dilute Anti-BrdU-POD, Fab fragments, stock solution (Solution 5) 1:100 with Washing buffer (Solution 2) supplemented with 10 mg/ml BSA (bovine serum albumin)*. ▪ For one 96-well microplate, dilute 100 µl Anti-BrdU-POD, Fab fragments, stock solution with 9.9 ml PBS and BSA (final concentration: 200 mU/ml).	⚠ ***Prepare shortly before use.***	Detect incorporated BrdU.
6	Peroxidase substrate	▪ Dissolve the ABTS powder (Bottle 7) in Substrate buffer (Bottle 6) and stir at +15 to +25°C to obtain a clear solution.	Store at +2 to +8°C for 2 months. ⚠ ***Keep protected from light.***	Visualization
6a	Peroxidase substrate containing Substrate enhancer	▪ If a low signal is expected, take an appropriate aliquot of substrate solution and add Substrate enhancer (Bottle 8), 1 mg/ml and dissolve by stirring for 15 minutes at +15 to +25°C. ▪ For one 96-well microplate, dissolve 10 mg Substrate enhancer in 10 ml Peroxidase substrate (Solution 6).	⚠ ***Prepare shortly before use. The Substrate solution containing Substrate enhancer is stable for only 4 hours.***	Improve signal.

2.2. Protocols

Assay protocol adherent cells

See section, **Working Solution** *for additional information on preparing solutions.*

1. Culture cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100 µl culture medium per well according to the media needs of the cells, in a humidified atmosphere, such as +37°C and 6.5% CO_2).
   The incubation period of the cell cultures depends on the particular experimental approach and the cell line used for the assay.

2. For most experimental setups, incubate cell cultures for 24 to 96 hours.

3. Add 10 µl BrdU labeling solution (Solution 1) per well (final concentration: 10 µM BrdU) and incubate for 2 to 18 hours at +37°C.
   For most applications, 2 to 6 hours is adequate. The optimal incubation time must be determined experimentally.

4. Using suction, carefully remove the culture medium containing the labeling solution.
   – Carefully wash cells twice with 250 µl Washing medium containing 10% serum per well.

5. After the last wash, carefully remove Washing medium and fix cells with 200 µl precooled fixative per well for 30 minutes at −15 to −25°C.

6. Carefully remove fixative and wash cells 3 times with 250 µl Washing medium containing 10% serum per well.

7. After the last wash, carefully remove Washing medium and incubate cells with 100 µl Nucleases, working solution per well for 30 minutes at +37°C.
   – Perform the nuclease incubation step in the absence of CO_2, for example, using a water bath.

8. Remove Nucleases, working solution (Solution 4a) and wash cells 3 times with 250 µl Washing medium containing 10% serum per well.

9. Remove Washing medium after the last wash and add 100 µl of Anti-BrdU-POD, Fab fragments, working solution (Solution 5a) per well; incubate for 30 minutes at +37°C.

10. Remove antibody conjugate and wash 3 times with 250 µl Washing buffer (Solution 2) per well.

11. Carefully remove Washing buffer after the last wash.
    – Add 100 µl Peroxidase substrate without (Solution 6) or with Substrate enhancer (Solution 6a) per well.
    – Incubate at +15 to +25°C for 2 to 30 minutes until positive samples show a green color and it is clearly distinguishable from the color of pure peroxidase substrate.

12. Measure extinction of the samples in a microplate reader at 405 nm with a reference wavelength of approximately 490 nm.

Additional information

- To obtain reliable results, perform all washing steps with PBS or cell culture medium containing 10% serum, such as FCS. Avoid washing cells with serum-free media or Washing buffer (Solution 2) before fixation.
- If for the initial incubation of the cells, a larger volume of culture medium is required, increase the amount of BrdU labeling solution correspondingly, for example, 20 µl BrdU labeling solution when cells are cultured in 200 µl medium.
- If the assay cannot be performed within one day, wash wells after fixation, as described. Remove Washing medium and store the microplates overnight without buffer at +2 to +8°C. To continue the assay, wash once with 250 µl Washing medium and proceed by adding the Nucleases, working solution (Solution 4a), as described.

Assay protocol suspension cells

See section, **Working Solution** *for additional information on preparing solutions.*

1. Culture cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100 µl culture medium per well according to the media needs of the cells, in a humidified atmosphere, such as +37°C and 6.5% CO_2).
   The incubation period depends on the particular experimental approach and on the cell line used for the assay.

2. For most experimental setups, incubate cells for 24 to 96 hours.

3. Add 10 µl BrdU labeling solution (Solution 1) per well to suspension cells grown in 100 µl culture medium (final concentration: 10 µM BrdU) and incubate for 2 to 18 hours at +37°C.
   For most applications, 2 to 6 hours is adequate. The optimal incubation time must be determined experimentally.

4. Carefully remove labeling medium using a cannula.
   – To avoid loss of cells, it is essential to spin down the cells for 10 minutes at 300 × *g* in a centrifuge which has a rotor device for microplates, before removing the labeling medium.
   Do not wash suspension cells prior to drying and fixation. Excess of BrdU will be readily removed by washing after fixation.

5. Let cells dry to the bottom of the microplate for approximately 2 hours at +60°C.

6. Fix cells with 200 µl precooled fixative per well for 30 minutes at −20°C.
   – After fixation, cells should be tightly attached to the bottom of the microplate.

7. Carefully remove fixative and wash cells 3 times with 250 µl Washing medium containing 10% serum per well.

8. After the last wash, incubate cells with 100 µl Nucleases, working solution (Solution 4a) per well for 30 minutes at +37°C.
   – Perform the nuclease incubation step in the absence of CO_2, for example, using a water bath.

9. Remove Nucleases, working solution and wash cells 3 times with 250 µl Washing medium containing 10% serum per well.

10. Remove Washing medium after the last wash and add 100 µl of Anti-BrdU-POD, Fab fragments, working solution (Solution 5a) per well and incubate for 30 minutes at +37°C.

11. Remove antibody conjugate and wash 3 times with 250 µl Washing buffer (Solution 2).

12. Carefully remove Washing buffer after the last wash.
    – Add 100 µl Peroxidase substrate without (Solution 6) or with Substrate enhancer (Solution 6a) per well.
    – Incubate at +15 to +25°C for 2 to 30 minutes until positive samples show a green color and it is clearly distinguishable from the color of pure peroxidase substrate.

13. Measure extinction of the samples in a microplate reader at 405 nm with a reference wavelength of approximately 490 nm.

Additional information

- To obtain reliable results, perform all washing steps with PBS or cell culture medium containing 10% serum, such as FCS. Avoid washing cells with serum-free media or Washing buffer (Solution 2) before fixation becaues it causes cell clumping.
- If for the initial incubation of the cells, a larger volume of culture medium is required, increase the amount of BrdU labeling solution correspondingly, for example, 20 µl BrdU labeling solution when cells are cultured in 200 µl medium.
- If the assay cannot be performed within one day, wash wells after fixation, as described. Remove Washing medium and store the microplates overnight without buffer at +2 to +8°C. To continue the assay, wash once with 250 µl Washing medium and proceed by adding the Nucleases, working solution (Solution 4a), as described.

Measurement of the proliferation of adherent cells AKR-2B

See section, **Working Solution** *for additional information on preparing solutions.*

To determine epidermal growth factor (EGF) activity, see Figure 1.

1. Seed 5.0 × 10^3 AKR-2B cells in 100 µl culture medium per well of a microplate (96 wells, tissue-culture grade, flat bottom).
2. Incubate cells for approximately 4 days at +37°C and 6.5% CO_2.
3. When cells are grown to confluency, carefully remove culture medium and add 200 µl Synchronization medium per well.
4. Incubate cells for 2 days at +37°C and 6.5% CO_2.
5. Carefully remove Synchronization medium and add 50 µl fresh Synchronization medium containing 0.5 µg/ml Insulin* per well.
6. Incubate for 2 hours at +37°C and 6.5% CO_2.
7. Add various amounts of recombinant human EGF* in a volume of 50 µl Synchronization medium containing 0.5 µg/ml insulin and 50 µg/µl BSA* (final concentration of EGF approximately 0.01 to 100 ng/ml).
8. Incubate for 18 hours at +37°C and 6.5% CO_2.
9. Add 10 µl BrdU labeling solution and incubate for 2 hours at +37°C and 6.5% CO_2.
10. Proceed as described in section, **Assay protocol adherent cells**.

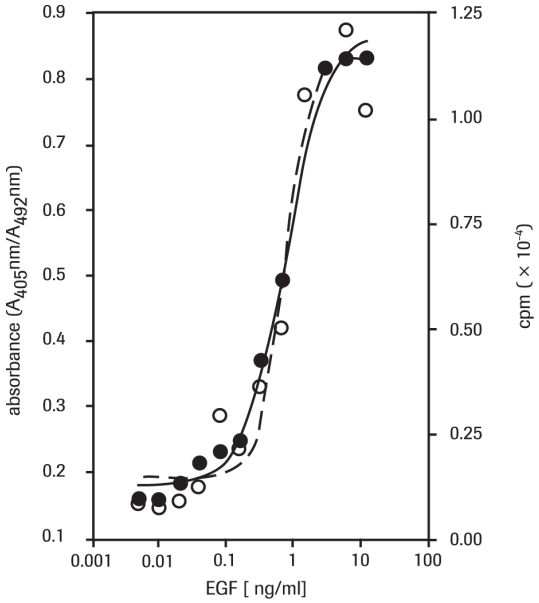

Fig. 1: Proliferation of AKR-2B cells (mouse fibroblast cell line) in response to recombinant human epidermal growth factor (hEGF) using the described protocol (●) or the [3H]-thymidine method (○, dashed line), respectively.

Measurement of the proliferation of suspension cells 7TD1

See section, **Working Solution** *for additional information on preparing solutions.*

To determine the activity of human interleukin-6 (hIL-6), see Figure 2.

1. Seed 7TD1 cells at a concentration of 4 × 10^3 cells/well in 100 µl culture medium containing various amounts of IL-6 (final concentration approximately 0.1 to 100 U/ml, 0.001 to 1 ng/ml) into microplates (tissue-culture grade, 96 wells, flat bottom).
2. Incubate cells for approximately 4 days at +37°C and 6.5% CO_2.
3. Add 10 µl BrdU labeling solution and incubate for 2 hours at +37°C and 6.5% CO_2.
4. Proceed as described in section, **Assay protocol suspension cells**.

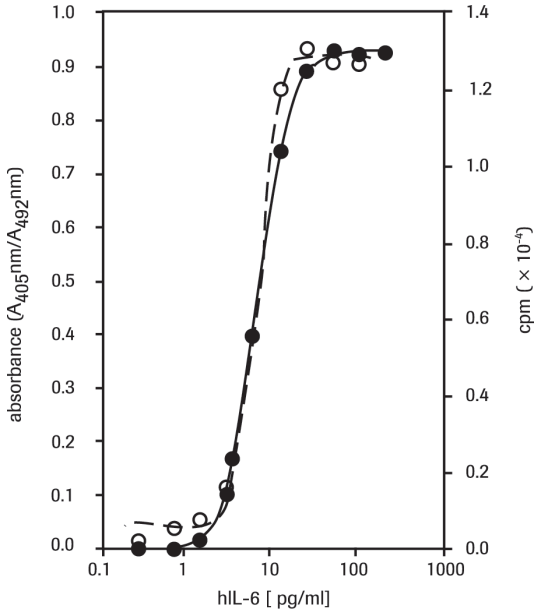

Fig. 2: Proliferation of 7TD1 cells (mouse-mouse hybridoma) in response to recombinant human interleukin-6 (hIL-6) using the described protocol (●) or the [3H]-thymidine method (○, dashed line), respectively.

2.3. Parameters

Sensitivity

Sensitivity is comparable to that of the traditional [^{3}H]-thymidine assay.

Specificity

Anti-BrdU-POD, Fab fragments specifically bind to 5-bromo-2′-deoxy-uridine and show cross-reactivity with 5-iodo-2′-deoxy-uridine (10%). The conjugate shows no cross-reactivity with 5-fluoro-2′-deoxy-uridine or any endogenous cellular component, such as thymidine or uridine. The antibody conjugate also binds to BrdU incorporated into cellular DNA.

3. Additional Information on this Product

3.1. Test Principle

Assay overview

The kit is based on the cell ELISA principle, see Figure 3.

1. Cells grown in a microplate are incubated with10 µM BrdU for approximately 2 to 18 hours.

2. Fixation of cells with 0.5 M ethanol/HCl.

3. Incubated with nucleases to partially digest the DNA and to improve the accessibility of the BrdU for the detection by antibodies.

4. Incorporated BrdU is detected with the monoclonal Anti-BrdU-POD, Fab fragments, and the bound conjugate is visualized with the soluble chromogenic substrate ABTS and measured using an ELISA reader.

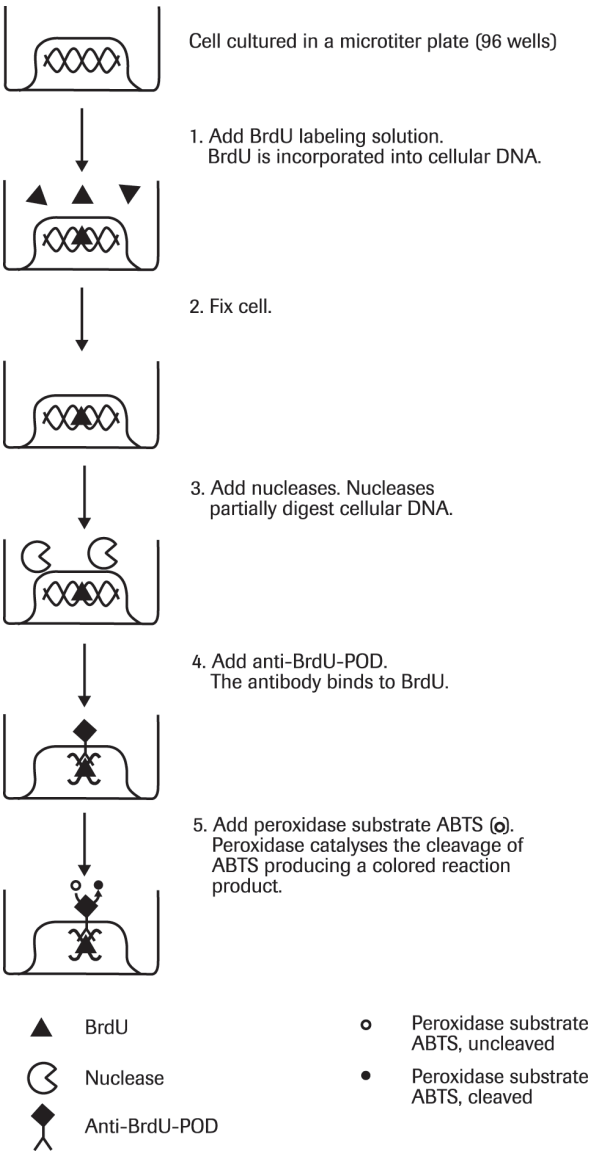

Fig. 3: Principle of the BrdU Labeling and Detection Kit III (POD).

Measurement of DNA synthesis

The determination of cellular proliferation, viability, and activation are key areas in a wide variety of cell biological approaches. The need for sensitive, quantitative, reliable, and automated methods led to the development of standard assays. For example, the incorporation of a radioactively labeled substance, such as [^{3}H] thymidine, or the release of a radioisotope, such as [^{51}Cr] after cell lysis is commonly used. Alternatively, the incorporation of 5-bromo-2′-deoxy-uridine (BrdU) in place of thymidine is monitored as a parameter for DNA synthesis and cellular proliferation in immunohisto- and cytochemistry and FACS analysis. Cells which have incorporated BrdU into DNA can be easily detected using a monoclonal antibody against BrdU and measured by an enzyme- or fluorochrome-conjugated second antibody.

How this product works

Monoclonal antibodies to BrdU are used to detect, both BrdU incorporated into tissue and single cells. Cell proliferation assays based on a microplate format are of particular importance for routine applications. Tetrazolium salts MTT and XTT are especially useful for the quantification of living metabolically active cells that are rapidly proliferating. Both, MTT* and XTT* work by being metabolized by mitochondrial dehydrogenases to form formazan dyes. Alternatively, the BrdU labeling technique is used to build a cell ELISA. This approach is particularly useful, when either a low number of population doublings or the stimulation of DNA synthesis is anticipated.

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

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

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Insulin, human	100 mg	11 376 497 001
Interleukin-6, human (hIL-6)	200,000 U, 2 µg, 1 ml	11 138 600 001
Cell Proliferation Kit I (MTT)	1 kit, 2,500 tests	11 465 007 001
Cell Proliferation Kit II (XTT)	1 kit, 2,500 tests	11 465 015 001
Epidermal Growth Factor, human (hEGF)	100 µg	11 376 454 001
Penicillin-Streptomycin	for 20 ml, 500x	11 074 440 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001

4.4. Trademarks

ABTS is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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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