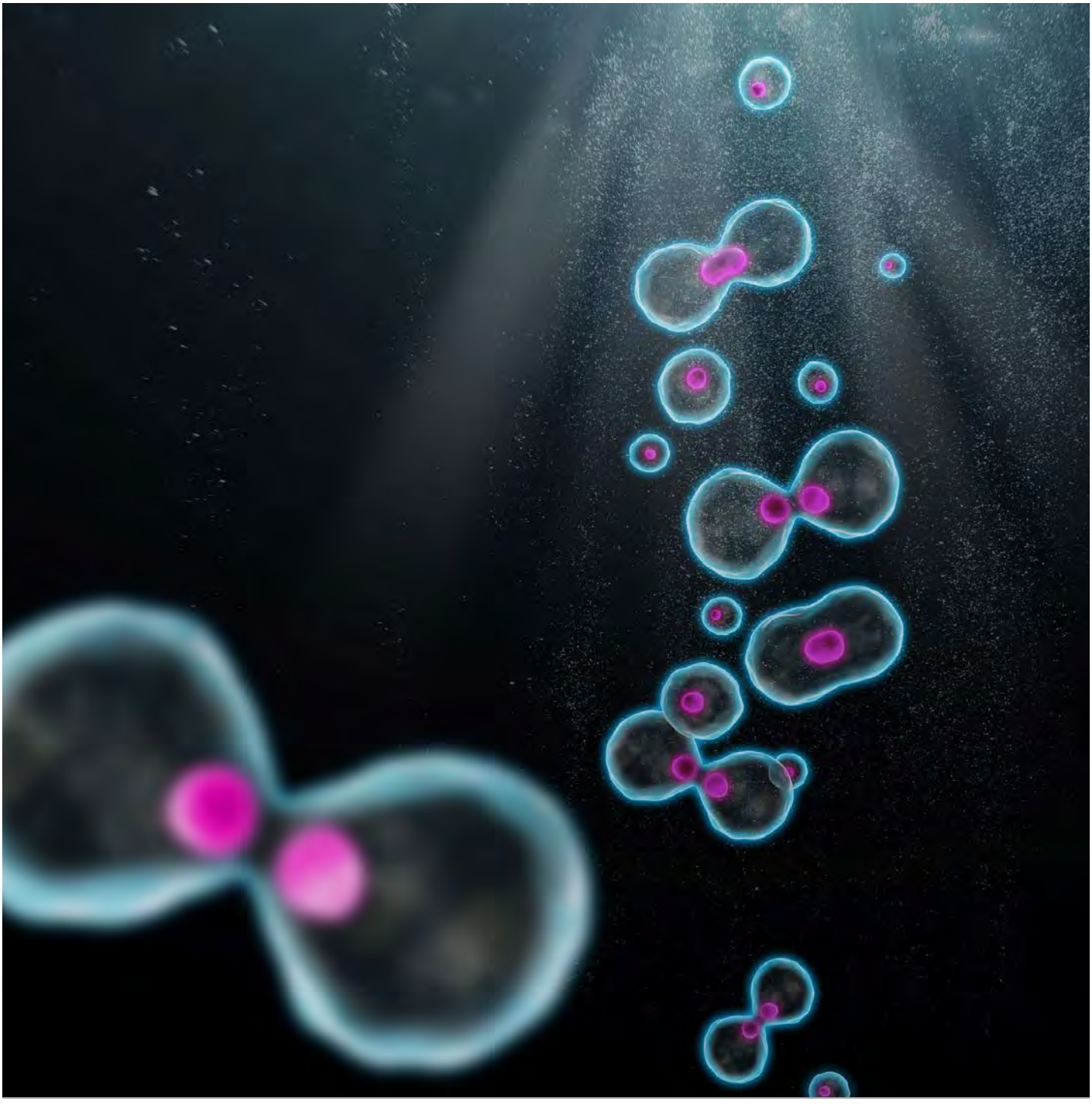
Cell Proliferation Products and Tips

Tools to Accurately Assess Proliferation



What Is Cell Proliferation?

Cell proliferation is the rapid expansion of a cell population due to growth and division. Proliferation has to be carefully regulated; even the smallest changes can result in an uncontrolled increase in cell numbers, and eventually lead to diseases like cancer.

Cell proliferation results from progression through the cell cycle divided into phases:

- Interphase (**G1**, **S**, and **G2**): in the gap 1 (G1) phase, the cell grows and cellular components are duplicated. During the synthesis (S) phase, the cell synthesizes a complete copy of the DNA. In the gap 2 (G2) phase, the cell prepares to divide by inducing metabolic changes that assemble the cytoplasmic components necessary for mitosis
- Mitotic (**M**) phase: during the M phase, nuclear division occurs, and the cell finally divides to create two identical daughter cells (Kapinas et al. 2013)

Under certain conditions, a cell can exit the cell cycle and enter a state of quiescence referred to as the gap 0 (G0) phase. This phase is, however, reversible and G0 cells can return to the G1 phase and resume growth and division if appropriately stimulated (Figure 1).

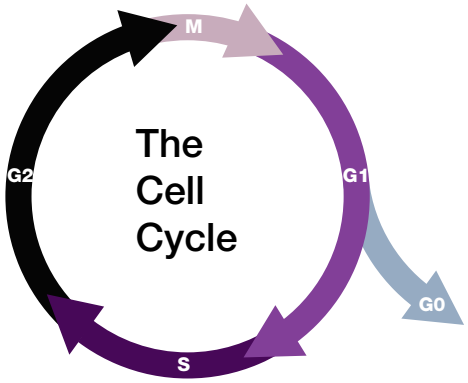


Fig. 1. Overview of the eukaryotic cell cycle. During cell division, cells pass through a series of stages collectively referred to as the cell cycle.

Our current understanding of the cell cycle and the key proteins involved has led to the development of a number of methods for quantifying and evaluating cell cycle processes and cell growth. These techniques are routinely applied in cancer and cell biology research. The most commonly used methods to assess dividing cells are highlighted here.

Measuring the Expression of Cyclins and CDKs

The master regulators of the cell cycle in eukaryotes are heterodimeric enzyme complexes, which consist of cyclins and cyclin-dependent kinases (CDKs) (Murray 2004). The distinct peak expression of cyclins and CDKs at various phases of the cell cycle can be exploited for cell cycle analysis. The total levels of individual cyclins, as well as their phosphorylated forms, can be detected using immunodetection methods such as western blotting (Figure 2A and 2B) or enzyme-linked immunosorbent assay (ELISA). Combining these techniques with assessment of DNA synthesis provides a thorough analysis of the cell cycle.

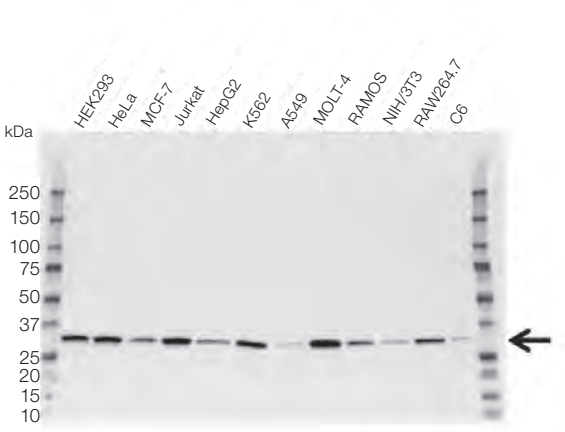


Fig. 2A. Western blot of whole cell lysates probed with Mouse Anti-CDK2 Antibody (Cat. #VMA00126).

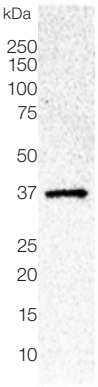


Fig. 2B. Western blot analysis of Molt-4 acute lymphoblastic derived T cell lysate probed with Mouse Anti-CDK6 Antibody (#VMA00022).

Table 1. Antibodies to study cyclins and CDKs.

Specificity	Application	Target	Reactivity	Clone	Catalog #
CDK1	WB	Human		Mouse Monoclonal (POH-1)	VMA00098
CDK1	WB	Human		Rabbit Polyclonal	VPA00762
CDK1	WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP2407
CDK2	WB	Human	Mouse, Rat	Mouse Monoclonal (1A6)	VMA00126
CDK2	WB	Human	Rat	Rabbit Polyclonal	VPA00748
CDK2 pThr160	WB, IHC-P	Human		Rabbit Polyclonal	AHP2570
CDK4	WB	Human		Mouse Monoclonal (1529CT850.162.73)	VMA00520
CDK4	WB	Human	Mouse, Rat	Mouse Monoclonal (DCS-31.2)	MCA1994
CDK4	WB	Human		Rabbit Polyclonal	VPA00749
CDK4	WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP2454
CDK5	WB	Human	Mouse, Rat	Mouse Monoclonal (AB04/3H6)	VMA00541
CDK5	WB	Human	Mouse	Rabbit Polyclonal	VPA00451
CDK6	IHC-F, IP, WB	Human		Mouse Monoclonal (DCS-83.1)	VMA00022
CDK6	WB, IHC-P	Human	Rat	Rabbit Polyclonal	AHP2455
CDK6 pTyr13	WB	Human		Rabbit Polyclonal	AHP2571
CDK6 pTyr24	WB, IHC-P	Human		Rabbit Polyclonal	AHP2572
CDK7	IHC-F, IP, WB	Human		Mouse Monoclonal (MO-1.1)	VMA00023
CDK9	E, IP, IHC-P, WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP315
Cyclin A	WB	Mouse	Human, Rat	Rabbit Polyclonal	AHP2408
Cyclin B1	WB, IHC-P	Human		Rabbit Monoclonal (RM281)	MCA6321
Cyclin D1	IHC-F, E, FC, IP, IHC-P, WB	Human	Rat	Mouse Monoclonal (CD1.1)	MCA1756
Cyclin D1	E, WB	Human	Mouse	Rabbit Polyclonal	VPA00892
Cyclin D1	WB	Human	Mouse, Dog	Rabbit Polyclonal	2407-0508
Cyclin D1	WB, IHC-P	Human	Bovine, Mouse, Rat	Rabbit Monoclonal (RM241)	MCA6301
Cyclin E	E, IP, WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP1245

Abbreviations: E, ELISA; FC, flow cytometry; IHC-F, immunohistology — frozen sections; IHC-P, immunohistology — paraffin sections; IP, immunoprecipitation; WB, western blotting.

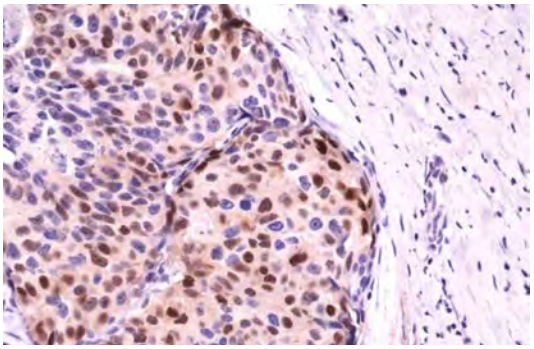


Fig. 2C. Immunohistochemical staining of formalin fixed and paraffin embedded human breast cancer tissue sections using Rabbit Anti-Cyclin D1 (#MCA6301).

Detecting Cell Proliferation Markers

Cell proliferation can also be measured by quantifying protein levels of key proliferation markers such as Ki-67, proliferating cell nuclear antigen (PCNA), and minichromosome maintenance 2 (MCM2), because of their distinct expression throughout the cell cycle. Antibodies specific to these proteins allow the distinction between actively dividing cells (positive expression) and quiescent (G0) cells (low or negative expression).

Ki-67 is a nuclear protein (Figure 3), that is present during all phases of the cell cycle (late G1, S, G2, and M) but absent in resting cells (G0 phase) (Gerdes et al. 1984). Its protein expression is low during G1 and early S phase, and gradually increases to reach a maximum during mitosis (Li et al. 2014). During this phase, Ki-67 is specifically expressed on the surface of chromosomes, and primarily involved in chromatin condensation (Cuylen et al. 2016, van Dierendonck et al. 1989). Ki-67 overexpression has been reported in many cancer types, including breast and lung, and this is associated with reduced patient survival (Pollack et al. 2004, Shiba et al. 2000, Stuart-Harris et al. 2008). As a result, Ki-67 has been used in the clinic as a prognostic and predictive marker for certain cancer types such as breast cancer (Li et al. 2014).

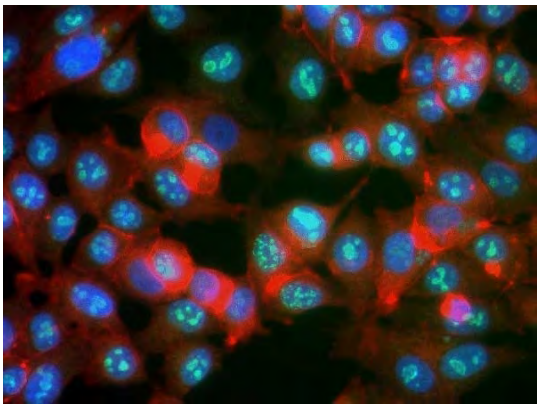


Fig. 3. IF analysis of MCF-7 with Human Anti-Ki-67 Antibody (#HCA006), green counterstained with phalloidin (red) and Hoechst (blue).

Table 2. Antibodies to study cell proliferation markers.

Specificity	Application	Target	Reactivity	Clone	Catalog #
Ki-67	E, IF, WB	Human	Rhesus Monkey, Bovine, Chimpanzee, Dog, Macaque	Human Recombinant Monoclonal (AbD2531)	HCA006
Ki-67	IHC-F, E, IHC-P, WB, IF	Human	Bovine, Macaque, Dog, Chimpanzee, Rhesus Monkey	Human Recombinant Monoclonal (AbD02531 _h/m_IgG1)	HCA053

Abbreviations: E, ELISA; IF, immunofluorescence; IHC-F, immunohistology — frozen sections; IHC-P, immunohistology — paraffin sections; WB, western blotting.

PCNA is an essential evolutionarily conserved protein, indicated by the observation that knocking out PCNA in mice results in embryonic lethality (Hu and Xiong 2006, Roa et al. 2008, Strzalka and Ziemienowicz 2011). The protein has been reported to play a role in various essential cellular processes such as DNA repair, chromatin remodeling, chromosome segregation, and cell cycle progression (Strzalka and Ziemienowicz 2011). PCNA has been described as the 'ringmaster of the genome' due to these diverse roles (Paunesku et al. 2001). It is primarily expressed in late G1 and S phases, decreases its expression in G2 and M phases, and is low or absent in G0 and early G1 phases (Kurki et al. 1986). Due to its role in replication and DNA repair, PCNA is considered a marker of cell proliferation in many cancers (Figure 4), including cervical cancer and gliomas (Lv et al. 2016).

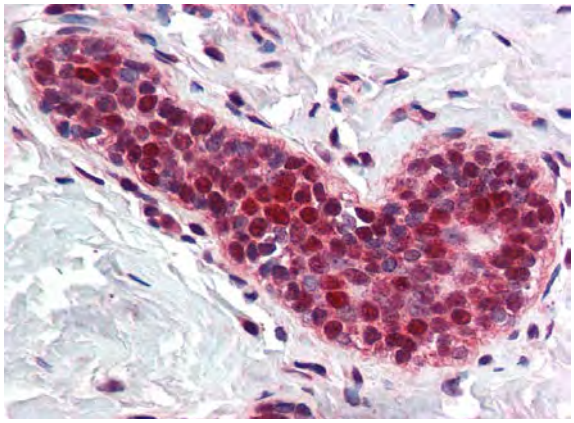


Fig. 4. IHC analysis of formalin fixed, paraffin embedded human breast with Rabbit Anti-PCNA (#AHP1419).

Table 3. Antibodies to study PCNA.

Specificity	Application	Target	Reactivity	Clone	Catalog #
PCNA	IHC-F, FC, IP, IHC-P, WB	Rat		Mouse Monoclonal (PC10)	MCA1558
PCNA	E, IHC-P, WB	Human	Chicken, Fish, Rat, Amphibia, Bovine, Monkey	Rabbit Polyclonal	AHP1419

Abbreviations: E, ELISA; FC, flow cytometry; IHC-F, immunohistology — frozen sections; IHC-P, immunohistology — paraffin sections; IP, immunoprecipitation; WB, western blotting.

Antibodies to Study MCM2

MCM2 is one of six members of the MCM protein family, which consists of MCM2-MCM7. These proteins play a key role in the initiation of DNA replication, by forming the pre-replication complex (pre-RC) in the G1 phase (Maiorano et al. 2006). The complex is critical for DNA replication in the subsequent S phase as it also stimulates the unwinding of the parental DNA strands (Maiorano et al. 2006, Labib et al. 2000). Compared to the other MCM family members, MCM2 is distinctly expressed throughout the cell cycle. MCM3-MCM7 proteins are not expressed in stoichiometric amounts and are regulated differently (Todorov et al. 1998).

MCM2 is highly expressed in early G1, moderately expressed in S, G2, and M phases, and absent during G0 (Maiorano et al. 2006). In addition, MCM2 demonstrates distinct cellular localization patterns that can be measured in cycling cells. It is expressed in the nucleus throughout the cell cycle, but is tightly bound to chromatin during G1 (Todorov et al. 1995). It is then displaced in the S phase and remains unbound in the G2 and M phases (Todorov et al. 1995).

The localization and expression pattern of MCM2 therefore makes it an ideal proliferation marker (Figure 5). It is also used as a diagnostic and prognostic marker in cancer types like kidney cancer and gliomas (Giaginis et al. 2010, Todorov et al. 1998).

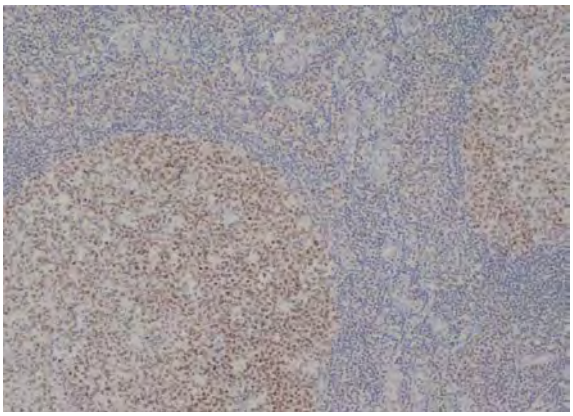


Fig. 5. IHC analysis of paraffin embedded human tonsil stained with Mouse Anti-MCM2 Antibody (#MCA1859) following citrate antigen retrieval.

Table 4. Antibodies to study MCM2.

Specificity	Application	Target	Reactivity	Clone	Catalog #
MCM2	WB	Human	Mouse, Rat	Mouse Monoclonal (OTI3B6)	VMA00259
MCM2	IHC-P, WB	Human		Mouse Monoclonal (CRCT2.1)	MCA1859
MCM2	IHC-F, IF, IHC-P, WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP2404

Abbreviations: IF, immunofluorescence; IHC-F, immunohistology — frozen sections; IHC-P, immunohistology — paraffin sections; WB, western blotting.

Assessing DNA Replication with Thymidine Analogs

The most common method of detecting proliferating cells, as well as assessing individual cell cycle phases, is by measuring newly synthesized DNA. Traditional methods use chemical compounds that are incorporated into DNA, instead of certain nucleotides. Thymidine analogs are the most commonly used.

5'-bromo-2'-deoxyuridine (BrdU) is a thymidine analog, which is incorporated into the newly synthesized DNA of proliferating cells instead of thymidine, when added to cell culture media. The incorporated BrdU can be detected and measured with the help of BrdU specific antibodies (Figures 6A and 6B). Other thymidine analogs, used for measuring DNA replication, include 5'-chloro-2'-deoxyuridine (CldU), 5'-iodo-2'-deoxyuridine (IdU), and 5'-ethynyl-2'-deoxyuridine (EdU) (Salic and Mitchison 2008, Tuttle et al. 2010).

Table 5. Antibodies to study BrdU.

Specificity	Application	Target	Clone	Catalog #
BrdU	FC, IF	Chemical	Monoclonal (AbD33758kg)	HCA320
BrdU	FC	Chemical	Monoclonal (AbD33761kg)	HCA321
BrdU	FC, IF	Chemical	Monoclonal (AbD33761kd)	HCA323
BrdU	FC	Chemical	Monoclonal (AbD33758kd)	HCA322
BrdU	FC	Chemical	Monoclonal (RF04-2)	MCA6143
BrdU	IF	Chemical	Monoclonal (RF06)	MCA6144
BrdU	IHC-F, FC, IHC, IF, IHC-P	Chemical	Mouse Monoclonal (Bu20a)	MCA2483
BrdU	FC, IHC-F, IF, IHC-P	Chemical	Rabbit Monoclonal	AHP2405

Abbreviations: FC, flow cytometry; IF, immunofluorescence; IHC-F, imunohistology — frozen sections; IHC-P, immunohistology — paraffin sections.

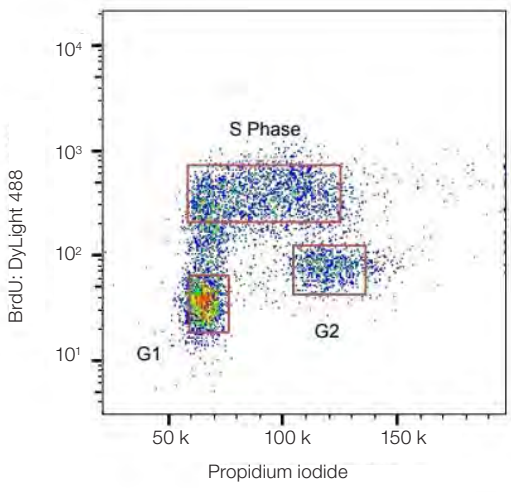


Fig. 6A. Flow analysis of BrdU labeled human lymphoma cells stained with Mouse Anti-BrdU Antibody (#HCA320).

Knowing the cross-reactivity of your anti-BrdU antibody is especially critical, when performing double-labeling experiments with BrdU and other nonhalogenated thymidine analogs (Table 6).

Table 6. Cross-reactivity results for flow cytometry.

Cat#	BrdU	CldU	EdU	IdU	Thymidine
AHP2405	+	+	-	+	-
HCA320	+	+	-	-	-
HCA321	+	+	-	-	-
HCA322	+	+	-	+	-
HCA323	+	+	-	-	-
MCA6143	+	+	+	+	-

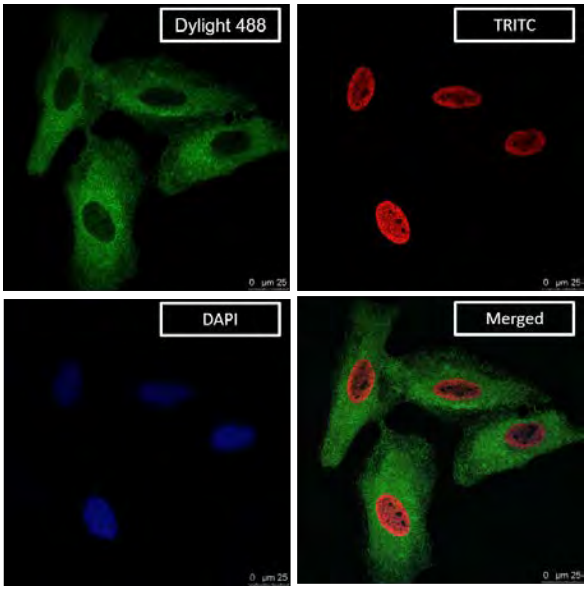


Fig. 6B. IF analysis of HeLa with Rat Anti-BrdU Antibody (#MCA6144).

CytoTrack Cell Proliferation Assays and CFDA-SE

CytoTrack Cell Proliferation Assays efficiently stain live cells to obtain excellent resolution of each cell division. CytoTrack Dyes enable you to measure cell proliferation in live cells using flow cytometry. As the labeled cells divide, the concentration of the dye is halved, and the proliferation can be measured based upon the reduced levels of fluorescence in subsequent generations. Using proprietary chemistry, the dye reacts with intracellular proteins and is retained in the cell with minimal efflux, allowing the CytoTrack Cell Proliferation Assays to track up to ten cell generations in dividing cells.

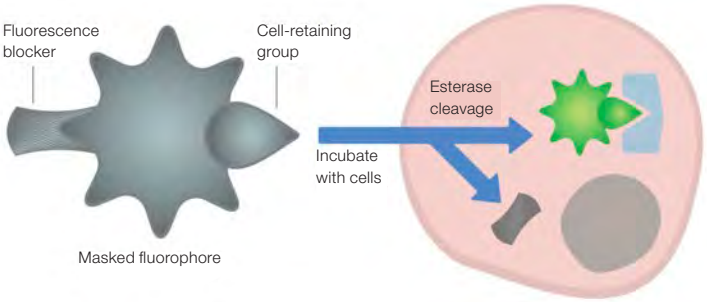


Fig. 7. Consisting of three components, CytoTrack Dyes efficiently label live cells for visualizing up to ten cell divisions.

The cell permeable CytoTrack Dye is initially nonfluorescent and becomes fluorescent as intracellular esterases cleave the fluorescence blocker moiety. The cell-retaining group of the CytoTrack Dye covalently binds to the intracellular proteins and remains inside the cell during cell division.

CytoTrack Cell Proliferation Dyes Key Benefits

- Nontoxic — minimal cellular toxicity
- Detection of intracellular targets — CytoTrack Dyes are compatible with standard formaldehyde-containing fixatives and saponin-based permeabilization buffers
- Suitability for multicolor cell analysis — a range of excitations and emissions available for compatibility with fluorescent proteins or fluorophore-labeled antibodies
- No washing required — use the cell proliferation assay directly with your cell culture media
- Suitability for long term tracking of labeled cells. Resolve up to 10 cell divisions compared with approximately 8 cell divisions with CFDA-SE

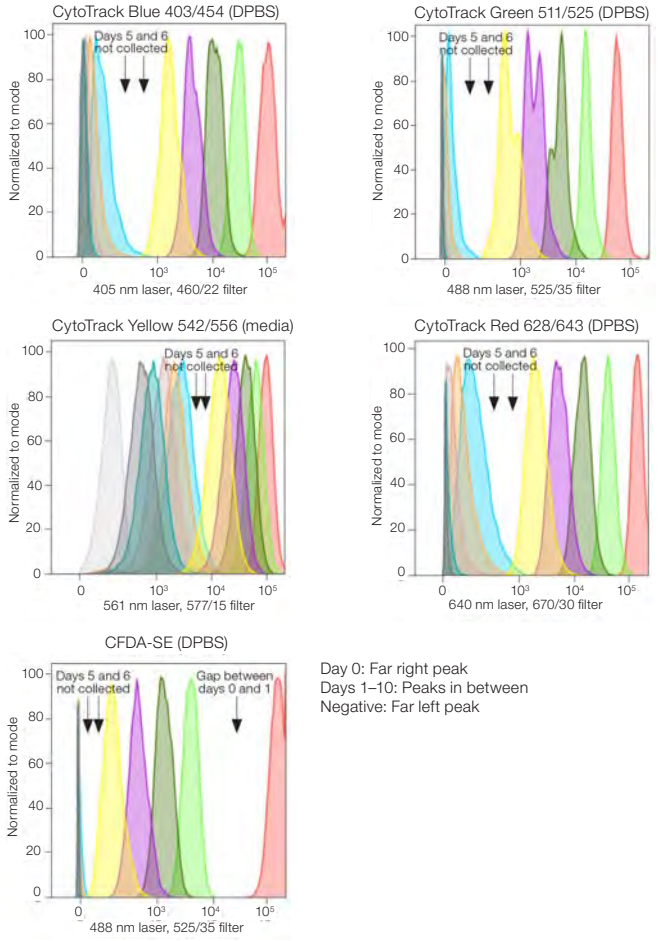


Fig. 8. CytoTrack Dyes and CFDA-SE performance on the ZE5 Cell Analyzer. 1x CytoTrack Dye solution in either cell culture media or buffer was added to 1 x 10^6 Jurkat cells/ml. Cells were incubated in the dark at room temperature for 15 min, then washed and resuspended with cell culture media or buffer. Half were run on the ZE5 Cell Analyzer (day 0) and the other half left to grow. Each day, half the sample was removed to run on the ZE5 Cell Analyzer and the culture media was replenished. DPBS, Dulbecco's phosphate buffered saline.

Table 7. CytoTrack Dyes properties.

Description	Excitation Max, nm	Emission Max, nm	Optimal Excitation Laser	Test Size	Catalog #
CytoTrack Blue	403	454	405	200 tests	1351202
CytoTrack Green	511	525	488	200 tests	1351203
CytoTrack Yellow	542	556	532	200 tests	1351204
CytoTrack Red	628	643	640	200 tests	1351205
CFDA-SE	494	521	488	500 µg	1351201

Please visit bio-rad-antibodies.com/CytoTrack for more information.

alamarBlue Reagent

Metabolic Proliferation Assay

alamarBlue Reagent is a cell viability assay reagent which contains the cell permeable, nontoxic, and weakly fluorescent blue indicator dye, resazurin. This is a trusted and established reagent which has been available since 1993. Resazurin is used as an oxidation-reduction (REDOX) indicator, that undergoes colorimetric change in response to cellular metabolic reduction. The reduced form, resorufin, is pink and highly fluorescent, and the intensity of fluorescence produced is proportional to the number of living cells respiring. alamarBlue Reagent is a direct indicator of cell health, by detecting the level of oxidation during respiration, quantitatively measuring cell viability and cytotoxicity.

Key advantages of alamarBlue include:

- Increased sensitivity — as few as 50 cells can be detected
- Excellent stability — allowing for continuous reading over several days in culture
- Safety — nontoxic to cells, users, and the environment

alamarBlue Reagent Online Calculator

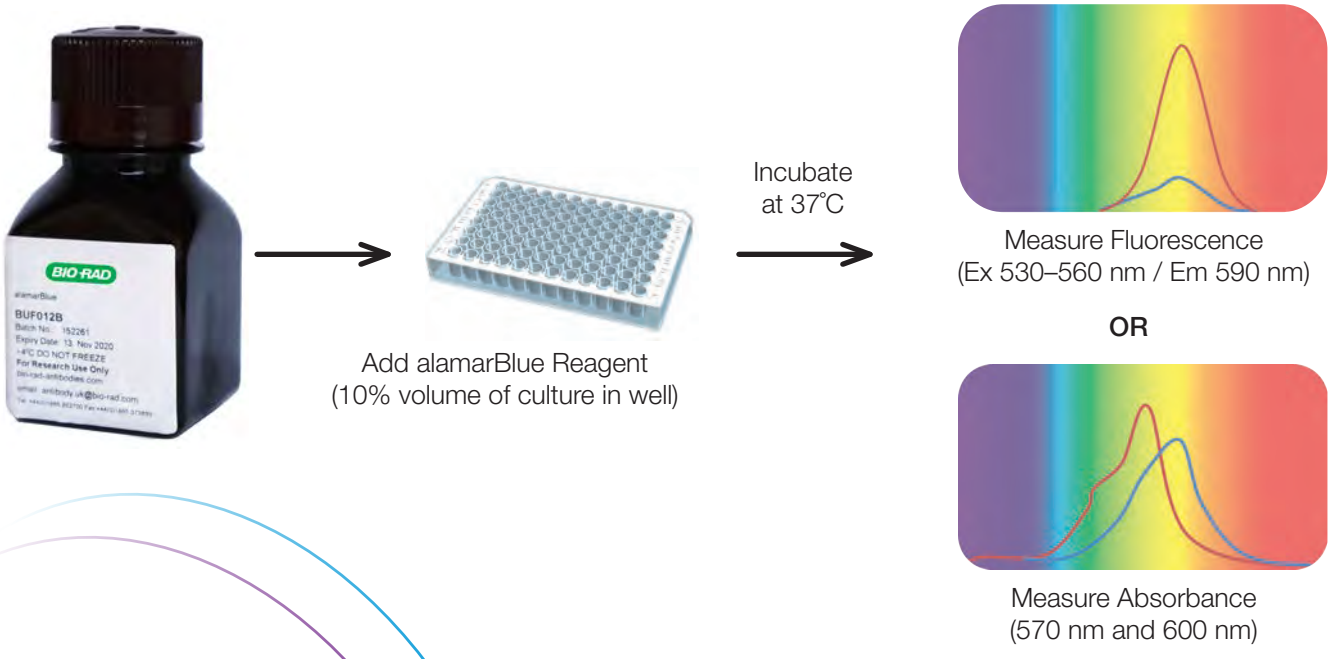
- Simple online colorimetric and fluorometric calculators
- Example calculations
- References
- FAQs
- Detailed protocols

bio-rad-antibodies.com/alamarblue

Table 8. alamarBlue reagents.

Pack Size	Usage	Catalog #
25 ml	Enough for 2,500 wells/96-well plate	BUF012A
100 ml	Enough for 10,000 wells/96-well plate	BUF012B

Note — calculations assume 100 µl final volume per well.



Antibodies to Study Other Key Cell Proliferation Markers

Table 9. Other key cell proliferation products.

Specificity	Application	Target	Reactivity	Clone	Catalog #
Aurora A	IF, IP, WB	Human	Mouse	Mouse Monoclonal (35C1)	MCA2249
Aurora A	WB	Human	Mouse	Rabbit Polyclonal	VPA00851
Aurora B	WB	Human	Mouse	Rabbit Polyclonal	VPA00485
Aurora B	E, WB	Human		Rabbit Polyclonal	AHP1261
Aurora B	WB, IHC-P	Human		Rabbit Monoclonal (RM278)	MCA6320
CENPF	WB	Human		Rabbit Polyclonal	VPA00837
CKII Alpha	IF, WB	Human		Mouse Monoclonal (3D9)	MCA3031Z
CKII Alpha	E, WB	Human		Recombinant Monoclonal (AbD05928)	HCA064
CHK1	WB	Human	Mouse, Rat	Mouse Monoclonal (2G1D5)	MCA6041
CHK1	WB	Human	Mouse, Rat	Rabbit Polyclonal	VPA00572
CHK1 pSer280	WB	Human		Rabbit Polyclonal	AHP2573
Kip1	WB	Human	Mouse, Rat	Mouse Monoclonal (KIP1/769)	VMA00657
Kip1	WB	Human	Mouse, Rat	Rabbit Polyclonal	VPA00721
Kip1 pThr187	WB	Human	Mouse, Rat	Rabbit Polyclonal	AHP2409
MAPK8	WB	Human	Rat	Mouse Monoclonal (GH05 /4G10)	VMA00407
MAPK8	IP, IHC-P, WB	Human		Mouse Polyclonal	AHP2322
MAPK8	IHC-P, WB	Human	Mouse	Rabbit Polyclonal	AHP2495
MAPK8 (pThr183/pTyr185)/MAPK10 (pThr221/pTyr223)	WB	Rat		Rabbit Polyclonal	AHP2693
p21	IP, WB	Human		Mouse Monoclonal (WA-1)	MCA2325
Survivin	IF, WB	Human		Mouse Monoclonal (5B10)	MCA4782Z
Survivin	E, WB	Human		Goat Monoclonal	AHP2075

Abbreviations: E, ELISA; IF, immunofluorescence; IHC-P, immunohistology — paraffin sections; IP, immunoprecipitation; WB, western blotting.

At Bio-Rad, we have developed a set of tips for your BrdU labeling experiments to ensure robust data generation.

Tips include:

1. Select the right BrdU antibody for your experiment.
2. Know your anti-BrdU antibody cross-reactivity.
3. Keep your BrdU fresh.
4. Perform a BrdU titration.
5. Optimize your DNA denaturation step.
6. Optimize your washing steps.
7. Carefully select your secondary antibody.
8. Include suitable controls.
9. Take tissue sample size into account when performing immunohistochemistry (IHC) experiments.
10. Consider spectral overlap in multichannel flow cytometry.

1. Select the Right BrdU Antibody for Your Experiment

Antibody selection is one of the most important considerations when planning any experiment. Ideally, your chosen antibody should have supporting data that shows characteristic positive staining of BrdU, in your chosen application. This can usually be found on the datasheet or product web page. For example, if you are analyzing your BrdU incorporation via flow cytometry, you should look for supplier data showing the characteristic "hook profile", when the nuclear dye is plotted on the X-axis and BrdU is plotted on the Y-axis. Using this profile, you should be able to discriminate between G1, S, and G2 phases. The cells that are in the S-phase of the cell cycle have brighter staining than those in G1 or G2 phase (Figure 9).

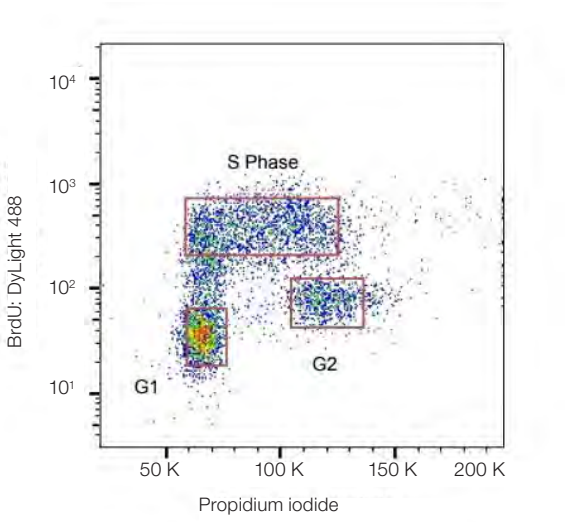


Fig. 9. Jurkat cells were treated with 100 µM BrdU and cells were stained with clone RF04-2 Rat Anti-BrdU Antibody (#MCA6143). As a secondary antibody, Rabbit F(ab')$_2$ Anti-Rat IgG:DyLight488 Conjugated Antibody (#STAR16D488GA) was used. Cells actively synthesizing DNA (S Phase) show BrdU incorporation, resutling in a bright signal from BrdU:DyLight488.

In addition, choose an anti-BrdU antibody with a different host species to the cells or tissue you plan to use for your experiment. For the best lot-to-lot consistency and long-term security of supply, use non-animal derived, recombinant anti-BrdU antibodies.

You should also perform titration experiments in order to determine the optimal concentration of your anti-BrdU antibody.

2. Know Your Anti-BrdU Antibody Cross-Reactivity

BrdU and thymidine are structurally similar. Their structural difference is illustrated in Figure 10; in the case of BrdU, the 5-methyl group (shown in blue) of thymidine has been substituted by bromine (highlighted in green) (Kolb et al. 1999, Sivakumar et al. 2004). As an alternative to bromine, the 5-methyl group of thymidine can be replaced with other halogens, such as chlorine (5'-chloro-2'-deoxyuridine, CldU) and iodine (5'-iodo-2'-deoxyuridine, IdU) (Sivakumar et al. 2004), to create different thymidine analogs.

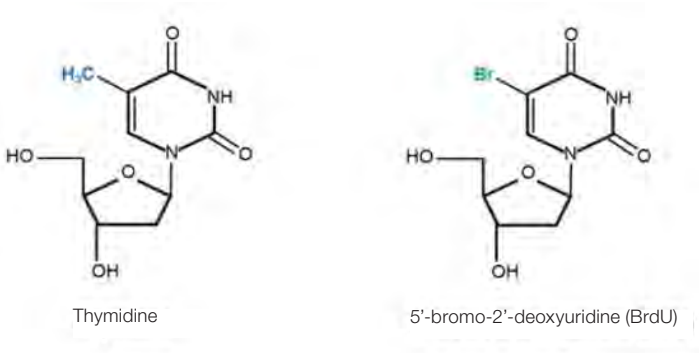


Fig. 10. Chemical structures of the DNA nucleoside thymidine and its analog BrdU. Structures adapted from Iball et al. (1966) (BrdU) and Young et al. (1969) (thymidine).

Anti-BrdU antibodies are likely to recognize other thymidine analogs due to their structural similarities. If you are looking to incorporate different analogs at different time points, for example when performing a dual pulse labeling experiment, check if your BrdU antibody has been tested for cross-reactivity with thymidine analogs.

It is also useful to look for data demonstrating a lack of cross-reactivity with thymidine. If your anti-BrdU antibody cross-reacts with thymidine in the DNA of cells, this could result in a false positive signal.

3. Keep Your BrdU Fresh

Add freshly prepared BrdU to appropriate growth media for your cells or tissue. Remember that the half-life of BrdU at 4°C is short, so make sure your BrdU stock solution is stored at -20°C.

4. Perform a BrdU Titration

The ideal concentration of BrdU and incubation time for optimal labeling depends on the rate of cell division, and needs to be optimized for your cells. Perform titration experiments to determine the concentration of BrdU that will give the best level of incorporation without inducing cytotoxicity.

5. Optimize Your DNA Denaturation Step

For successful staining, it is important to include a DNA denaturation step to allow the antibody access to the incorporated BrdU. One common approach uses depurination and cleavage of DNA by acids such as hydrochloric acid. Other treatments with copper ions, heat, nucleases, and sodium hydroxide have also been reported (Liboska et al. 2012, Kennedy et al. 2000). Optimize your denaturation step, by establishing the hydrochloric acid concentration, temperature, and incubation period that give the best BrdU detection and keep the protein structure in the cells intact.

6. Optimize Your Washing Steps

After the denaturation step, ensure you perform sufficient washing in an appropriate amount of wash buffer. Any remaining acid that is not washed away could denature your antibody and affect the ability to detect BrdU. Keep in mind that the wash steps also need to be optimized for the anti-BrdU antibody to give the best signal-to-background ratio.

7. Carefully Select Your Secondary Antibody

If you are planning to detect your anti-BrdU antibody with a secondary antibody, rather than a directly conjugated anti-BrdU antibody, background staining may be reduced by using a secondary antibody raised in a different species to the target cells/tissues.

8. Include Suitable Controls

Proper controls add meaning to your data. You should make sure that all the relevant controls are included. These could be:

Negative controls: to observe any effect of the solvent on your cells, treat a sample of cells with only the solvent that the stock BrdU solution is diluted in. For example, if your stock BrdU solution is diluted in dimethyl sulfoxide (DMSO), you should prepare negative control cells treated with DMSO only. It is essential that the volume of solvent administered to your negative control group is the same as the volume of BrdU in DMSO administered to your treatment group, and that the incubation period is identical for both samples.

Isotype controls: you should also consider isotype controls. This involves staining a sample of your cells or tissue with an antibody that binds to an unrelated target (not designed to target BrdU), but is of the same isotype as the antibody targeting BrdU.

Secondary only antibody controls: if you are using secondary antibodies, it is advisable to run samples that are assayed with just the secondary antibody (without using the primary BrdU antibody), to check for background staining that results from nonspecific binding of the secondary antibody.

9. Take Your Tissue Sample Size into Account While Performing IHC Experiments

BrdU staining of tissue samples can be detected using immunohistochemistry. Note, the antibodies detecting BrdU need to penetrate all cells within a fixed tissue, so the size of your tissue sample will affect BrdU staining. You can overcome this by sectioning the tissue or by prolonging antibody incubation (Salic and Mitchison 2008).

10. Consider Spectral Overlap in Multichannel Flow Cytometry

If you use flow cytometry to analyze BrdU incorporation, remember to select a nuclear dye that has no spectral overlap with the fluorophore chosen for the BrdU antibody. One simple way to check this is to use a spectraviewer tool.

Visit bio-rad-antibodies.com/spectraviewer to access Bio-Rad's free spectraviewer tool.

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