Technical Data Sheet

488 EdU Click Proliferation Kit

Product Information

Material Number: **565455**
Size: 50 Tests
RRID: AB_2869677

Description

The BD Pharmingen™ 488 EdU Click Proliferation Kit provides a tool for analyzing DNA synthesis in cycling cells. In this method, the thymidine analog EdU (5-ethynyl-2´-deoxyuridine) is incorporated into newly synthesized DNA by cells progressing through the S (DNA synthesis) phase of the cell cycle. EdU can then be detected in fixed and permeabilized cells with a fluorophore-labeled azide. The reaction of the fluorophore-labeled azide and EdU occurs via a copper-catalyzed click reaction between the azide moiety of the fluorophore and the alkyne moiety of EdU. When co-stained with a DNA dye such as 7-AAD, PI, or DAPI, cell populations may be segmented by flow cytometry into the G0/G1-phases (2N DNA content, EdU-negative), S-phase (2N-4N DNA content, EdU-positive), or G2/M-phases (4N DNA content, EdU-negative).

The BD Pharmingen™ 488 EdU Click Proliferation Kit contains 6-FAM Azide, which is excited by the blue laser and has an excitation maximum of 496 nm and an emission maximum of 516 nm.

Please note that this kit is provided as Part 1 of 2 (Components A, B, and C) to be stored dry and protected from light at -20°C, and Part 2 of 2 (Components D, E, F, and G) to be stored dry at 2 - 8°C or room temperature.

Kit Contents

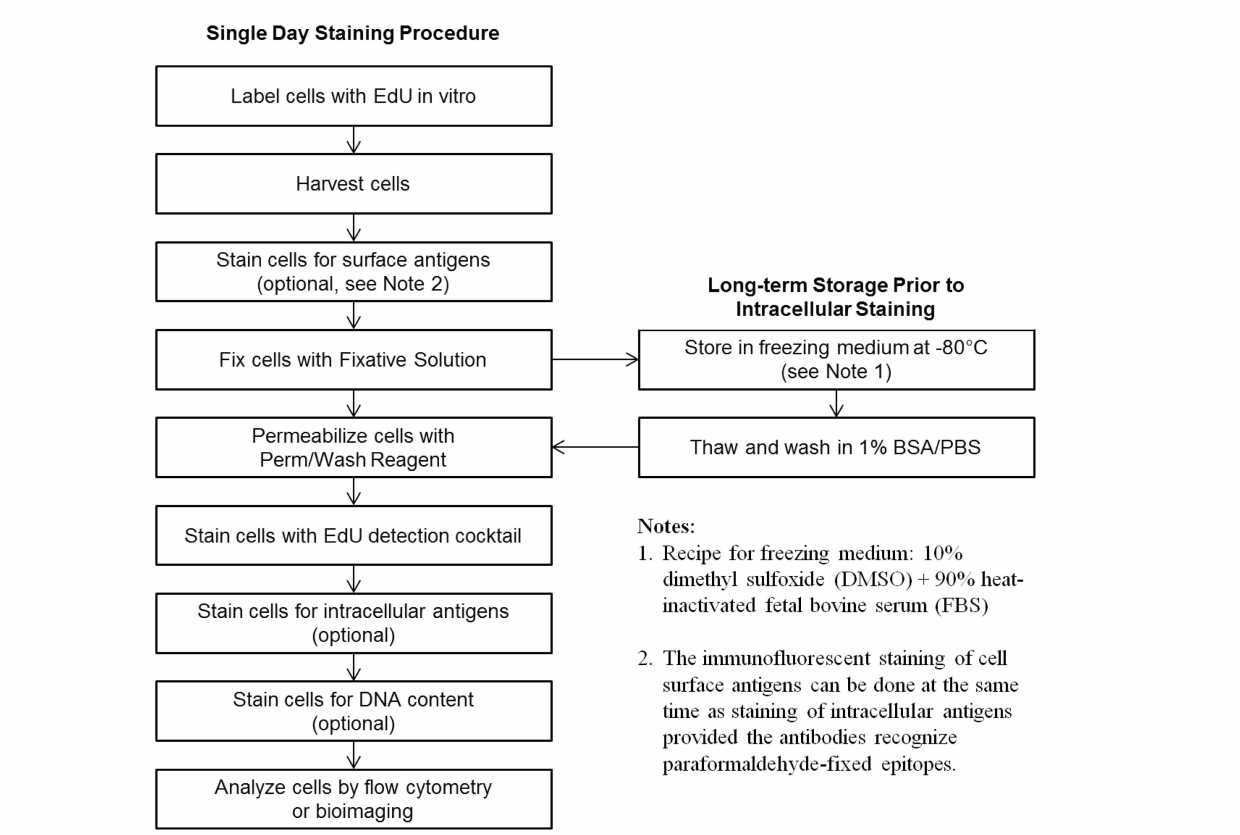
Component ID	Component Description	Amount	Long Term Storage Conditions
A	EdU (5-ethynyl-2´-deoxyuridine)	10 mg	-20°C, dry
B	6-FAM Azide (10 mM)	130 µL	-20°C, dry, dark
C	Buffer Additive (10×)	400 mg	-20°C, dry
D	Saponin-based Permeabilization and Wash Reagent (10×)	50 mL	2 - 8°C
E	Fixative Solution (4% paraformaldehyde-based)	5 mL	2 - 8°C
F	Catalyst Solution	2 mL	RT, dry
G	DMSO	5 mL	RT, dry

Please note that Hazard Warnings for components listed above are found on Page 5 of this document.

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**



Application Notes

Application

Intracellular staining (flow cytometry)	Tested During Development
Immunocytochemistry	Tested During Development
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

A. Materials Required but Not Provided

- Cells of interest
- FACS tubes or imaging plates/slides
- Buffered saline solution, such as PBS, DPBS, or TBS
- BD Pharmingen™ Stain Buffer (BSA) (Cat. No. 554657) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656)
- Deionized water
- Fluorescent antibodies or dyes for immunophenotypic or functional analysis (optional)
- DNA content dye (optional)

B. Cytometry Requirements

Blue laser-equipped cytometers (BD FACSCanto™ II, BD LSRFortessa™, BD LSR™ II, and BD Accuri™ C6 Flow Cytometer) can be used. 6-FAM can be read out of filters commonly used for FITC or Alexa Fluor® 488 (eg, 530/30 bandpass filter). Fluorescence compensation is best achieved using a sample of the cells of interest.

EdU incorporation is best analyzed with logarithmic amplification, while total DNA content is best analyzed with linear amplification. If DNA content is to be assessed, samples should be acquired on a low flow rate to generate the best resolution of 2N and 4N populations.

C. Preparation of Stock Solutions

1. Allow all vials to warm to room temperature before opening.
2. *Component A:* For the preparation of a 10 mM stock solution of EdU, add 4 mL DMSO or 1× PBS to EdU and mix until completely dissolved. After use, store any remaining solution at -20°C. When stored as directed, this stock solution is stable for up to one year. We recommend preparing aliquots to avoid repeated freeze-thaw cycles.

3. *Component B:* For 6-FAM azide, we recommend preparing aliquots to avoid repeated freeze-thaw cycles. Aliquots should be stored dry and protected from light at -20°C.
4. *Component C:* For the preparation of a 10× stock solution of the buffer additive, add 4 mL deionized water to the buffer additive and mix until completely dissolved. After use, store any remaining solution at -20°C. When stored as directed, this stock solution is stable for up to 6 months. If the solution starts to develop a brown color, it has degraded and should be discarded. We recommend preparing aliquots to avoid repeated freeze-thaw cycles.
5. *Component D:* For the preparation of 500 mL of 1× saponin-based permeabilization buffer and wash reagent, add 50 mL Component D to 450 mL 1% BSA in 1× PBS. After use, store any remaining solution at 2 - 8°C.

D. Recommended Staining Procedure - Flow Cytometry

Labeling of Cells with EdU

1. Add desired amount of EdU to cells in culture medium. We have found a final concentration of 10 µM EdU to be sufficient for labeling of human and mouse cell lines and primary cell populations.
    a. During addition of EdU to cells in culture, avoid disturbing the cells in any way (eg, centrifugation steps or temperature changes) that may disrupt the normal cell cycling patterns. The cell culture density should not exceed 2 × 10^6 cells/mL.
2. Incubate the treated cells for the desired length of time. Different cell types may require different incubation periods for optimal labeling with EdU. As a starting point, we recommend 10 µM EdU for 1 hour.
3. Harvest cells, pellet by centrifugation, and remove EdU-containing media.
4. Dislodge the cell pellet, resuspend cells in BD Pharmingen™ Stain Buffer (BSA) (Cat. No. 554657) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) at 1 × 10^7 cells/mL, and aliquot 1 × 10^6 cells per 12 × 75-mm FACS tube.
5. If you do not wish to stain for surface antigens, add 1 mL BD Pharmingen™ Stain Buffer, pellet cells by centrifugation, remove the supernatant, and proceed to step D9.

Staining of Cell Surface Antigens (Optional)

6. Add fluorescent antibodies specific for cell surface markers to tubes and vortex gently to mix.
    a. Because the click reaction cocktail contains copper and copper is known to quench the fluorescence of R-PE, antibody conjugates containing R-PE or R-PE tandems should be stained after the click reaction (Staining of Intracellular and/or Surface Antigens, steps D19-22).
7. Incubate samples for 30 minutes at room temperature or on ice.
8. Wash samples by adding 1 mL BD Pharmingen™ Stain Buffer, pelleting by centrifugation, and removing the supernatant.

Fixing and Permeabilizing the Cells

9. Dislodge the cell pellet and resuspend cells in 100 µL Fixative Solution (Component E) per tube.
10. Incubate the cells for 15 minutes at room temperature.
11. Wash samples by adding 1 mL BD Pharmingen™ Stain Buffer, pelleting by centrifugation, and removing the supernatant.
12. Dislodge the cell pellet, resuspend the cells in 100 µL of 1× Saponin-Based Permeabilization Buffer (prepared in step C5), and vortex gently to mix.

Detection of EdU Incorporation

13. Prepare the assay cocktail as described in Table I after section G, adding reagents in the same order indicated in the table. If the ingredients are not added in the order listed, the reaction may not proceed optimally or might fail. Once the assay cocktail is prepared, use it within the next 15 minutes.
    a. Please note that in some cases, titration of the dye azide (Component B) can increase resolution of EdU positive and negative populations. In this case, dilute an aliquot of Component B with an appropriate amount of DMSO and add 1 µL of the diluted dye azide in place of the concentrated stock.
14. Add 500 µL of the assay cocktail to each sample and vortex gently to mix.
15. Incubate the samples for 30 minutes at room temperature protected from light.
16. Wash samples by adding 2 mL 1× Saponin-Based Permeabilization Buffer (prepared in step C5), pelleting by centrifugation, and removing the supernatant.
17. Repeat step D16.
18. If you do not wish to stain for intracellular antigens or DNA content, dislodge the cell pellet and resuspend cells in 500 µL 1× PBS or equivalent, and proceed to analysis on the flow cytometer.

Staining of Intracellular and/or Surface Antigens (Optional)

19. Dislodge the cell pellet and resuspend cells in 100 µL BD Pharmingen™ Stain Buffer.
20. Add fluorescent antibodies specific for intracellular and/or surface markers to tubes and vortex gently to mix.
    a. Because the click reaction cocktail contains copper and copper is known to quench the fluorescence of R-PE, antibody conjugates containing R-PE or R-PE tandems should be stained at this point in the procedure.
21. Incubate samples for 30 minutes at room temperature or on ice.
22. Wash samples by adding 1 mL BD Pharmingen™ Stain Buffer, pelleting by centrifugation, and removing the supernatant.

Staining for Total DNA Content (Optional)

23. Dislodge the cell pellet and resuspend in 500 µL 1× PBS containing an appropriate amount of DNA dye (eg, DAPI, PI, or 7-AAD) for your cell type of interest. Vortex gently to mix.
24. Proceed to analysis by flow cytometry.

E. Recommended Staining Procedure - Bioimaging

This assay was optimized for 96-well plate imaging. Reagent volumes may need to be optimized for other imaging geometries.

Labeling of Cells with EdU

1. Culture desired cell type in well-plates.
2. Add desired amount of EdU to cells in culture medium, or replace culture medium with fresh, pre-warmed medium containing desired amount of EdU. We have found a final concentration of 10 µM EdU to be sufficient for labeling of human and mouse cell lines and primary cell populations.
    a. During addition of EdU to cells in culture, avoid disturbing the cells in any way (eg, centrifugation steps or temperature changes) that may disrupt the normal cell cycling patterns. The cell culture density should not exceed 70% confluence.
3. Incubate the treated cells for the desired length of time. Different cell types may require different incubation periods for optimal labeling with EdU.
4. Remove EdU-containing media, wash cells once in BD Pharmingen™ Stain Buffer (BSA) (Cat. No. 554657) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and remove wash buffer. If you do not wish to stain for surface antigens, proceed to step E8.

Staining of Cell Surface Antigens (Optional)

5. Prepare fluorescent antibodies specific for cell surface markers in BD Pharmingen™ Stain Buffer, and add to samples. Stain volume should be large enough to cover the well entirely (eg, at least 50 µL for a 96-well).
6. Incubate samples for 60 minutes at room temperature.
7. Wash samples twice with BD Pharmingen™ Stain Buffer, and remove wash buffer.

Fixing and Permeabilizing the Cells

8. Add Fixative Solution (Component E) to each sample. Fixative Solution volume should be large enough to cover the well entirely (eg, at least 50 µL for a 96-well).
9. Incubate samples for 15 minutes at room temperature.
10. Wash samples twice with BD Pharmingen™ Stain Buffer, and remove wash buffer.
11. Add 1× Saponin-Based Permeabilization Buffer (prepared in step C5) to each sample. Permeabilization Buffer volume should be large enough to cover the well entirely (eg, at least 50 µL for a 96-well).
12. Incubate samples for 15 minutes at room temperature.

Detection of EdU Incorporation

13. Make up a working solution of the dye azide (Component B) by diluting 1:5 with DMSO (eg, dilute 1 µL dye azide with 4 µL DMSO). Vortex well to mix.
14. Prepare the assay cocktail as described in Table II after section G, adding reagents in the same order indicated in the table. If the ingredients are not added in the order listed, the reaction may not proceed optimally or might fail. Once the assay cocktail is prepared, use it within the next 15 minutes.
    a. Please note that in some cases, titration of the dye azide (Component B) can increase resolution of EdU positive and negative populations. In this case, dilute the working solution of dye azide prepared in step E13 with an appropriate amount of DMSO and add 1 µL of the diluted working solution in place of the concentrated working solution.
15. Add 100 µL of the assay cocktail to each sample, on top of the 50 µL 1× Saponin-Based Permeabilization Buffer added in step E11. If necessary, more cocktail may be used per sample, but all components should be kept in the same ratios and added in the same order in order for the reaction to proceed optimally.
16. Incubate the samples for 30 minutes at room temperature, protected from light.
17. Wash samples twice with 1X Saponin-Based Permeabilization Buffer (prepared in step C5), 5 minutes per wash, and remove wash buffer.
18. If you do not wish to stain for intracellular antigens or DNA content, add PBS to each sample and proceed to imaging. PBS volume should be enough to completely cover the wells entirely (eg, at least 50 µL for a 96-well).

Staining of Intracellular and/or Surface Antigens (Optional)

19. Prepare fluorescent antibodies specific for intracellular and/or surface markers in BD Pharmingen™ Stain Buffer, and add to samples. Stain volume should be large enough to cover the well entirely (eg, at least 50 µL for a 96-well).
20. Incubate samples for 60 minutes at room temperature.
21. Wash samples twice with BD Pharmingen™ Stain Buffer, and remove wash buffer.

Staining for Total DNA Content (Optional)

22. Add 100 µL 1× PBS containing an appropriate amount of DNA dye (eg, DAPI or 7-AAD) for your cell type of interest.
23. Proceed to analysis by bioimaging.

F. Notes

1. The click reaction cocktail contains copper, which is known to quench the fluorescence of R-PE. Antibody conjugates containing PE or R-PE should be stained after the click reaction.
2. The copper present in the click reaction cocktail may affect binding of anti-GFP antibodies to their epitopes. We recommend staining with anti-GFP antibodies before the click reaction.
3. Organic dyes (eg, Alexa Fluor® dyes), PerCP-Cy™5.5, APC, Brilliant™ Violet, and Brilliant™ Ultraviolet dyes have been found to be compatible with the click reaction.
4. Large shifts in autofluorescence can occur from exposure to the click component cocktail. We recommend that all controls, including unstained and single stained controls, also be treated with the click component cocktail with or without the dye azide as appropriate so that control and test samples exhibit the same autofluorescent properties.

G. Hazards identification

Warning: *Component B, 6-FAM Azide (10 mM), Material Number 51-9012109, contains 99.6% DMSO.*

Hazard statements

Combustible liquid.

Precautionary statements

Keep away from flames and hot surfaces. - No smoking.
Wear protective gloves / eye protection.
Wear protective clothing.
In case of fire: Use for extinction: CO2, powder or water spray.
Store in a well-ventilated place. Keep cool.

Danger: *Component E, Fixative Solution, Material Number 51-9012105, contains 4% formaldehyde.*

Hazard statements

Harmful if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalation.
May cause respiratory irritation.

Precautionary statements

Do not breathe mist/vapours/spray.
Wear protective clothing / eye protection.
Wear protective gloves.
If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If skin irritation or rash occurs: Get medical advice/attention.
Dispose of contents/container in accordance with local/regional/national/international regulations.

Component F, Catalyst solution, Material Number 51-9012106, contains 1.6% copper sulfate.

Hazard statements

Harmful to aquatic life with long lasting effects.

Precautionary statements

Avoid release to the environment.
Dispose of contents/container in accordance with local/regional/national/international regulations.

Warning: *Component G, Material Number 51-9012107, contains DMSO.*

Hazard statements

Combustible liquid.

Precautionary statements

Keep away from flames and hot surfaces. - No smoking.
Wear protective gloves / eye protection.
Wear protective clothing.
In case of fire: Use for extinction: CO2, powder or water spray.
Store in a well-ventilated place. Keep cool.

Table I. Flow Cytometry Component Cocktail

Material	Component	Number of Assays				
		1	2	3	5	10
PBS, DPBS, or TBS	Not provided	439 µL	878 µL	1.317 mL	2.195 mL	4.390 mL
Catalyst Solution	**F**	10 µL	20 µL	30 µL	50 µL	100 µL
Dye Azide	**B**	1 µL	2 µL	3 µL	5 µL	10 µL
Buffer Additive (prepared in step C4)	**C**	50 µL	100 µL	150 µL	250 µL	500 µL
Total Volume	-	500 µL	1 mL	1.5 mL	2.5 mL	5 mL

Table II. Bioimaging Component Cocktail

Material	Component	Number of Assays				
		1	2	3	5	10
PBS, DPBS, or TBS	Not provided	87 µL	174 µL	261 µL	435 µL	870 µL
Catalyst Solution	**F**	2 µL	4 µL	6 µL	10 µL	20 µL
Dye Azide (prepared in step E13)	**B**	1 µL	2 µL	3 µL	5 µL	10 µL
Buffer Additive (prepared in step C4)	**C**	10 µL	20 µL	30 µL	50 µL	100 µL
Total Volume	-	100 µL	200 µL	300 µL	500 µL	1000 µL

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
550825	PI/RNase Staining Buffer	100 mL	(none)
556463	Propidium Iodide Staining Solution	2 mL	(none)
564907	DAPI Solution	1 mg	(none)
558609	Alexa Fluor® 647 Rat anti-Histone H3 (pS28)	100 Tests	HTA28
559925	7-AAD	2 mL	(none)

Figure 1

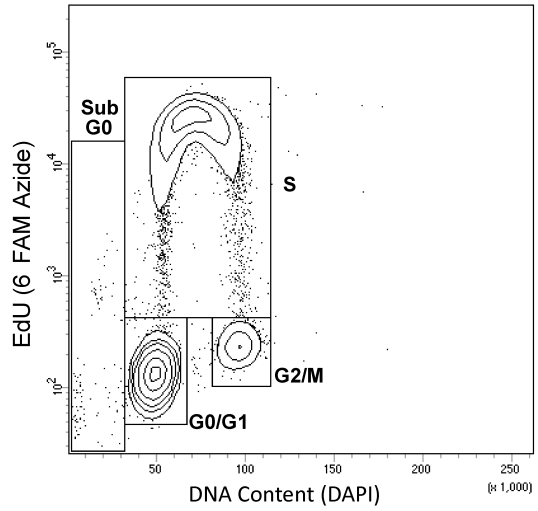


Figure 2

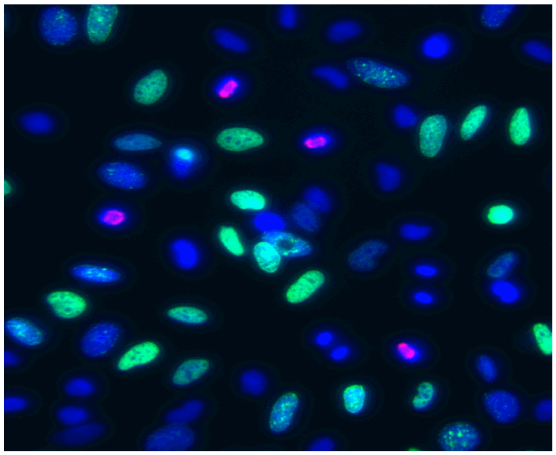


Figure 1: Cell Cycle Analysis of Proliferating HeLa cells. *HeLa cells were pulsed with 10 µM EdU for 1 hour, and then fixed, permeabilized, and stained with 6-FAM azide and BD Pharmingen™ DAPI Solution (Cat. No. 564907) according to the recommended assay procedure. Histograms were derived from gated events based on light scattering characteristics of intact HeLa cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Flow Cytometry System.*

Figure 1: Fluorescent Staining of HeLa Cells for EdU Incorporation and Histone H3 (pS28). *HeLa cells were seeded in a 96-well imaging plate at ~15,000 cells per well. After overnight incubation, cells were pulsed with 10 µM EdU for 1 hour, and then fixed, permeabilized, and stained with 6-FAM azide (pseudo-colored green) according to the Recommended Assay Procedure. Cells were washed twice and stained with Alexa Fluor® 647 Rat Anti-Histone H3 (pS28) (pseudo-colored red, Cat. No. 558609). Nuclei were counterstained with DAPI (pseudo-colored blue). 6-FAM appears cyan when colocalized with DAPI, and Alexa Fluor® 647 Rat Anti-Histone H3 (pS28) appears magenta when colocalized with DAPI. Images were captured on a BD Pathway™ 435 Bioimager System with a 20X objective and merged using BD Attovision™ software.*

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Limited Use Label License — Research Use Only: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform research, and not for any clinical, diagnostic, vaccine, protective, prophylactic, preventive or therapeutic use in humans, animals, or plants.
5. Alexa Fluor™ is a trademark of Life Technologies Corporation.

References

Buck SD, Bradford J, Gee KR, Agnew BJ, Clarke ST, Salic A. Detection of S-phase cell cycle progression using 5-ethynyl-2'-deoxyuridine incorporation with click chemistry, an alternative to using 5-bromo-2'-deoxyuridine antibodies. *Biotechniques*. 2008; 44(7):927-929. (Methodology)

Cavanagh BL, Walker T, Norazit A, Meedeniya AC. Thymidine analogues for tracking DNA synthesis. *Molecules*. 2011; 16(9):7980-7993. (Methodology)

Kolb HC, Finn MG, Sharpless KB. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew Chem Int Ed Engl*. 2001; 40(11):2004-2021. (Methodology)

Kotogany E, Dudits D, Horvath GV, Ayaydin F. A rapid and robust assay for detection of S-phase cell cycle progression in plant cells and tissues by using ethynyl deoxyuridine. *Plant Methods*. 2010; 6(1)(Methodology)

Salic A, Mitchison TJ. A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc Natl Acad Sci U S A*. 2008; 105(7):2415-2420. (Methodology)

Wang Q, Chan TR, Hilgraf R, Fokin VV, Sharpless KB, Finn MG. Bioconjugation by Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition. *J Am Chem Soc*. 2003; 125(11):3192-3193. (Methodology)