# Bucculite™ iFluor® 555 Protein Synthesis Assay Kit *Red Fluorescence*

Catalog number: 22352
Unit size: 100 Tests

| Component | Storage | Amount (Cat No. 22352) |
|---|---|---|
| Component A: Bucculite™ AHA | Freeze (< -15 °C), Minimize light exposure | 1 vial (20 µL, 25 mM DMSO solution) |
| Component B: Bucculite™ iFluor® 555 dye | Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component C: DMSO | Freeze (< -15 °C) | 1 vial (100 µL) |

## OVERVIEW

Bucculite™ iFluor® 555 Protein Synthesis Assay Kit provides a sensitive and non-radioactive method for detecting nascent protein synthesis through metabolic amino acid labeling. The assay utilizes AHA (L-azidohomoalanine), a methionine analog containing an azide functional group that is incorporated into newly synthesized proteins during active translation. The incorporated AHA residues are fluorescently detected using Bucculite™ iFluor® 555 dye to generate a red fluorescence signal corresponding to de novo protein synthesis activity. The kit is compatible with standard TRITC/Texas Red® filter sets and supports fluorescence imaging and high-content analysis workflows. Unlike many commercially available protein synthesis detection kits that rely on copper-catalyzed click chemistry reactions, the Bucculite™ detection workflow avoids copper-mediated labeling conditions while supporting sensitive fluorescent detection of metabolically labeled proteins. Bucculite™ iFluor® 555 Protein Synthesis Assay Kit can be used to study translational regulation, cellular stress responses, and the effects of protein synthesis inhibitors or other treatments on nascent protein production.

## AT A GLANCE

1. Add Bucculite™ AHA working solution (1X) in live growing cells
2. Incubate cells for 30 minutes under optimal conditions for the cell type
3. Perform cell fixation and permeabilization
4. Add Bucculite™ iFluor® 555 dye working solution on fixed cells
5. Incubate cells for 30 minutes at room temperature
6. Counterstain with nuclear dye (Optional)
7. Add PBS and acquire image with fluorescence microscope using Cy3 filter set

Allow vials to completely thaw and warm to room temperature before opening. Prior to use, briefly centrifuge reagents to maximize the reagent recovery.

## KEY PARAMETERS

**Fluorescence microscope**

| | |
|---|---|
| Emission | Cy3 filter set |
| Excitation | Cy3 filter set |
| Recommended plate | Black wall/clear bottom |

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**Bucculite™ iFluor® 555 dye stock solution (1000X):**

Add 10 µL of DMSO (Component C) to the vial of Bucculite™ iFluor® 555 dye vial (Component B) to make 100X Bucculite™ iFluor® 555 dye stock solution.

## PREPARATION OF WORKING SOLUTION

**Bucculite™ AHA working solution (1X):**

Prepare a working stock solution of Bucculite™ AHA (Component A) by diluting 1:500 in prewarmed L-methionine-free medium for a 50 µM final working solution.

**Bucculite™ iFluor® 555 dye working solution (1X):**

Make 1X working solution by adding 1 µL of Bucculite™ iFluor® 555 dye stock solution into 1 mL of PBS or buffer of your choice and mix well.

**Note:** Prepare the working solutions fresh and use them within 2 hours.

**Note:** 10 mL of working solution is enough for 100 tests.

## SAMPLE EXPERIMENTAL PROTOCOL

1. Plate cells at desired density and allow cells to recover overnight before additional treatment.
2. Treat cells as desired.
3. Remove the treatment and add 100 µL of Bucculite™ AHA working solution (1X) per well.
4. Incubate cells for 30 minutes under optimal conditions for the cell type.
5. Remove the Bucculite™ AHA working solution (1X) and wash cells twice with PBS.
6. Add 100 µL per well of 4% formaldehyde (AAT Cat# 20010, ReadiUse™ 4% formaldehyde fixation solution) in PBS.
7. Incubate cells for 20 minutes at room temperature.
8. Remove fixative and wash cells twice with PBS.
9. Add 100 µL per well of 0.5% Triton X-100 in PBS.
10. Incubate cells for 20 minutes at room temperature.
11. Remove the permeabilization buffer and wash cells twice with PBS.
12. Add 100 µL per well of Bucculite™ iFluor® 555 dye working solution (1X).
13. Incubate cells for 20 minutes at room temperature, protect from light.
14. Remove dye staining solution and wash cells twice with PBS.
15. **Optional:** Counterstain cells with nuclear stain (AAT Cat# 17548, Nuclear Blue DCS1) as necessary.
16. Add PBS and acquire image with fluorescence microscope using Cy3 filter set.

## EXAMPLE DATA ANALYSIS AND FIGURES

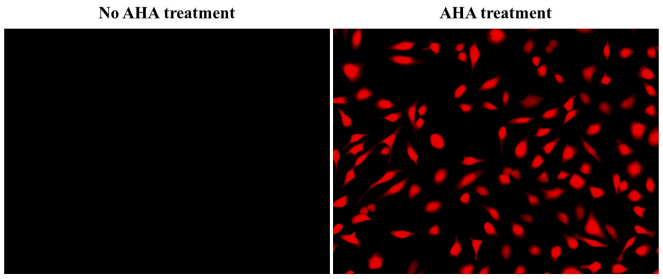


**Figure 1.** Detection of nascent protein synthesis using Bucculite™ iFluor® 555 Protein Synthesis Assay Kit (Cat. #22352). Fluorescence microscopy images of HeLa cells without AHA treatment (left panel) or treated with Bucculite™ AHA (right panel). Incorporated AHA was detected using Bucculite™ iFluor® 555 dye (red). AHA-treated cells showed increased red fluorescence signal corresponding to active de novo protein synthesis, while untreated cells exhibited minimal background fluorescence.

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