TC-FlAsH™ and TC-ReAsH™ reagents

LIVE-CELL IMAGING AND PROTEIN LABELING TOOLS.

The versatility of biarsenical reagents

Since Roger Tsien and colleagues first described the use of biarsenical reagents for the site-specific labeling of proteins in live cells in 1998, many applications for these reagents have been described in the literature (Table 1). Most applications describe the use of FlAsH and ReAsH reagents for labeling specific proteins in a live-cell context (references 1, 3–6, 8, 10–15) (Figure 1), while other publications describe the use of biarsenical technology in applications as varied as affinity purification, SDS-PAGE, and protease assays (references 2, 7, 9). Such extensive literature coverage indicates that FlAsH and ReAsH reagents represent a powerful and flexible labeling strategy for protein labeling.

How FlAsH and ReAsH work

The biarsenical labeling technology works through the high-affinity interaction of arsenic with thiols. The FlAsH reagent is essentially fluorescein that has been modified to contain two arsenic atoms at a set distance from each other (Figure 2A). Similarly, the ReAsH reagent is based on resorufin that has been modified to contain two appropriately spaced arsenic atoms. These virtually nonfluorescent reagents (shown in Figure 2B in an ethanedithiol (EDT)-bound state) become highly fluorescent upon binding to a tetracysteine (TC) sequence (Figure 3). The most commonly used TC sequence is the hexapeptide Cys-Cys-Pro-Gly-Cys-Cys; binding of the FlAsH or ReAsH reagent to this TC tag forms four covalent bonds, and the binding is reversible through competition with thiol-reducing agents. Thus, the method basically comprises incorporating a TC tag into a protein of interest, followed by detection with the green-fluorescent FlAsH or red-fluorescent ReAsH reagent.

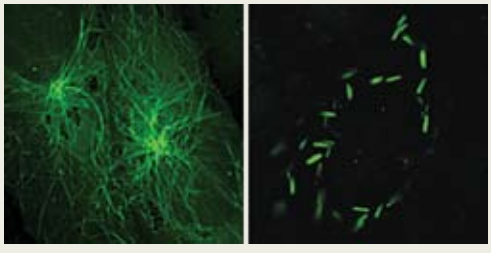

Figure 1—CHO-K1 cells expressing a tetracysteine-tagged version of β-tubulin labeled with TC-FlAsH™ reagent. Upon treatment with vinblastine, a compound known to perturb cytoskeletal structure, tubulin drastically rearranges from a reticular structure (A) to rod shaped (B).

One approach, many advantages

Several site-specific fluorescence tagging strategies have been developed for protein detection, including GFPs, Halo-tag, and SNAP-tag. Although fluorescent proteins remain the gold standard for live-cell imaging experiments due to their autofluorescent properties, exogenous fluorophore tagging strategies offer unique advantages. Using expression tags, researchers can add different fluorophores to change the color of a fusion protein, with no need to perform subcloning. Researchers can also base affinity purification strategies around the tag, attach nonfluorescent ligands such as biotin or a solid support, and utilize the tag for protein detection in SDS-PAGE (Figure 4).

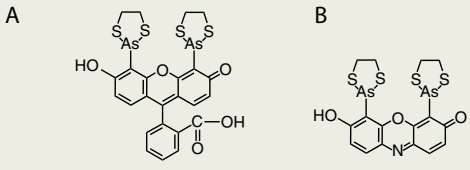

Figure 2—The structures of FlAsH-EDT$_2$ (A) and ReAsH-EDT$_2$ (B) biarsenical labeling reagents.

TC-FlAsH™ and TC-ReAsH™ reagents

Exemplifying the exogenous tagging method, Molecular Probes® TC-FlAsH™ and TC-ReAsH™ reagents are designed to provide optimum performance in the most challenging protein detection applications. Our TC-FlAsH™ and TC-ReAsH™ reagents encompass the full power of the technology, offering:

- a small tag size for minimal disruption of native protein structure/function
- very strong and reversible labeling
- extremely sensitive fluorogenic detection

For more information about these reagents and their use, visit our website at **probes.invitrogen.com**. ■

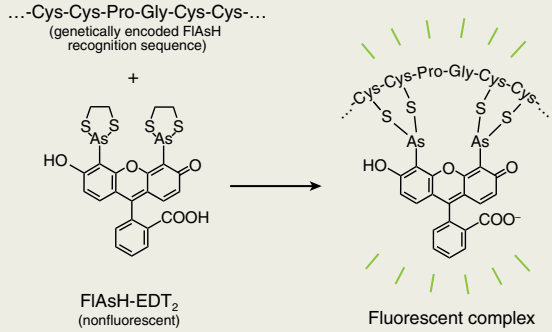

Figure 3—Schematic diagram of the fluorogenic nature of biarsenical reagents. Binding of the nonfluorescent FlAsH-EDT_2 reagent to a tetracysteine sequence yields a highly fluorescent complex.

Table 1—Publications describing unique applications of biarsenical reagents.

Year	Journal	Authors	Application	Reference
1998	*Science* 281:269	Griffin, B.A. et al.	Original description of FlAsH as a tool for site-specific labeling of proteins in live cells	1
2000	*Protein Sci* 9:213	Thorn, K.S. et al.	Affinity purification of TC-tagged proteins	2
2001	*Nat Biotechnol* 19:321	Rice, M.C. et al.	Experimental system for assessing gene repair using FlAsH in *Saccharomyces cerevisiae*	3
2002	*J Am Chem Soc* 124:6063	Adams, S.R. et al.	New blue and red biarsenical ligands	4
2002	*Science* 296:503	Gaietta, G. et al.	Correlation of electron microscopy to fluorescence microscopy	5
2003	*Nat Biotechnol* 21:1505	Tour, O. et al.	Genetically targeted chromophore-assisted light inactivation (CALI) using ReAsH	6
2004	*Electrophoresis* 25:2447	Feldman, G. et al.	In-gel detection of TC-tagged proteins using FlAsH	7
2004	*Nat Neurosci* 7:244	Ju, W. et al.	Pulse-chase experiments and trafficking of AMPA receptors using FlAsH and ReAsH	8
2005	*Anal Biochem* 336:75	Blommel, P.G.	Fluorescence anisotropy assay for protease activity of TC-tagged proteins	9
2005	*Nat Methods* 2:171	Hoffmann, C. et al.	A FlAsH-based FRET approach to determine GPCR activation in living cells	10
2005	*Nat Methods* 2:959	Enninga, J. et al.	Labeling of TC-tagged proteins in live bacteria	11
2005	*Nat Biotechnol* 23:1308	Martin, B.R. et al.	Optimization of the biarsenical-binding TC motif for mammalian cells	12
2006	*Biotechniques* 41:569	Estevez, J.M. and Somerville, C.	Live-cell imaging of synthetic peptides expressed in plants	13
2006	*J Am Chem Soc* 128:12040	Spagnuolo, C.C. et al.	Improved photostable FRET-competent TC probes	14
2006	*Nat Methods* 3:817	Arhel, N. et al.	Four-dimensional tracking of intracellular HIV complexes	15

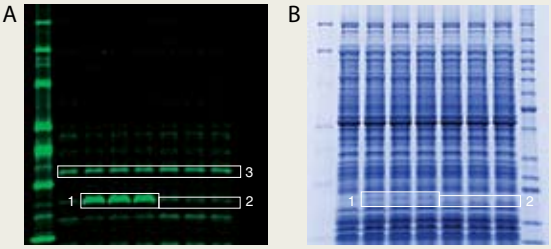

Figure 4—Protein detection using FlAsH reagent in polyacrylamide gels. FlAsH reagent was included in the sample loading buffer of TC-tagged acyl carrier protein (ACP) and untagged ACP in *E. coli* extract and subjected to SDS-PAGE. A fluorescent image was obtained directly after electrophoresis without the need for any gel staining or destaining (A). The gel was subsequently stained with SimplyBlue™ SafeStain and is shown for comparison (B). Box 1 highlights the TC-tagged ACP after 20, 60, and 180 minute *in vitro* protein synthesis reactions. Box 2 corresponds to untagged ACP. Box 3 is an endogenous *E. coli* protein that contains a naturally occurring tetracysteine sequence that allows FlAsH to bind.

Product	Quantity	Cat. no.
TC-FlAsH™ II In-cell Tetracysteine Tag Detection Kit *green fluorescence* *for live-cell imaging*	1 kit	T34561
TC-ReAsH™ II In-cell Tetracysteine Tag Detection Kit *red fluorescence* *for live-cell imaging*	1 kit	T34562
TC-FlAsH™ TC-ReAsH™ II In-cell Tetracysteine Tag Detection Kit *with mammalian TC-Tag Gateway® expression vectors* *green fluorescence* *red fluorescence*	1 kit	T34563