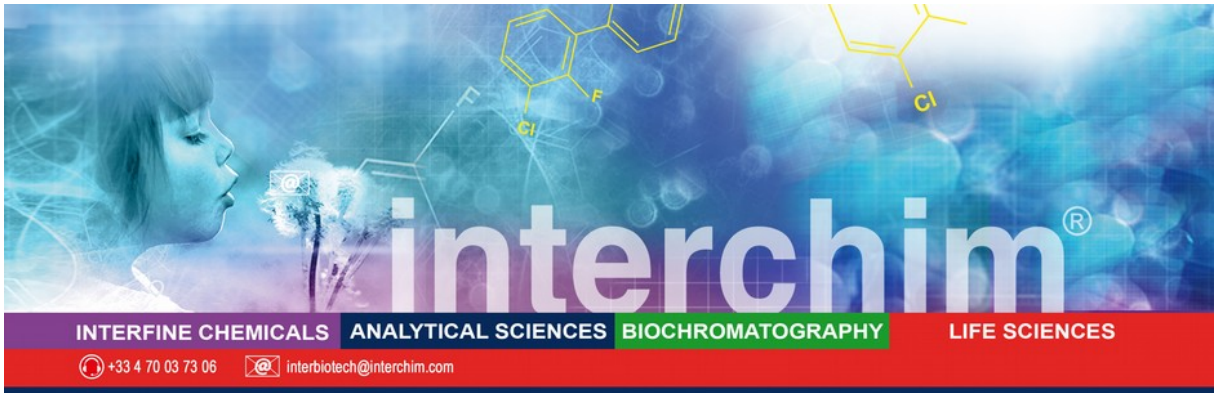
FT-AM376A



Fluorescent MTS

To study real-time monitoring of conformational changes, since fluorophores coupled to introduced cysteines can change their fluorescence during a conformational change.

Product Information

Product name cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$. max. (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Comment
AMCA - MTS FP-AM364A, 5 mg	370.45	353 / 442	19 000	7-amino-4-methylcoumarin-3-acetic acid is one of the most widely used UV-excitable, blue fluorescent dye
ABD - MTS FP-CP7020, 10 mg	352.41	376 / 510	8 000	• A pH dependent thiol reactive MTS-Benzoxadiazole fluorophore derived from ABD-F. • Combines the fast and selective reactivity of the MTS functionality with the fluorescence properties of ABD-F.
Dansyl - MTS FP-CP7030, 10 mg	388.53	335/526	4 400	• Reacts specifically and rapidly with thiols to form mixed disulfides. • Used to probe the structures of the ACh receptor channel, the GABAA receptor channel, and lactose permease.
Fluorescein -4- MTS FP-R59301, 10 mg	513.54	492 / 515	75 000	• Fluorescence lifetime may yield information regarding distances and molecular motion in a protein molecule.
CR110 - MTS FP-AM367A, 5 mg	511.58	502 / 524	77 000	• Green fluorescent thiol-reactive dye. CR110 (5-(and-6)- carboxyrhodamine 110) • Its photostability is far better than that of the fluorescein. Thus, CR110-MTS is a superior green fluorescent dye for labeling thiols.
CR110 –lc- MTS FP-AY800A, 5 mg	624.74	502 / 524	77 000	Similar to CR110-MTS except that it has a X spacer molecule between the dye and the MTS reactive group. The linker molecule may enhance fluorescence when the dye is attached to proteins.
Rhodamine 6G - MTS FP-AM376A, 5 mg	595.74	520 / 546	105 000	Fast-reacting and highly selective thiol-reactive rhodamine dye
TAMRA - MTS FP-60222A, 5 mg	567.69	540 / 565	95 000	Popular carboxytetramethylrhodamine
ROX - MTS FP-58296A, 5 mg	671.84	568 / 595	78 000	Mixed isomers (MTS-5(6)-carboxy-X-rhodamine)
SR101 - MTS FP-AM379A, 5 mg	743.94	583 / 603	112 000	Sulforhodamine 101
MTSET FP-U0351A, 100 mg	278.23			Positively charged MTS reagent with fast-reacting and highly selevtive thiol-reactive compounds

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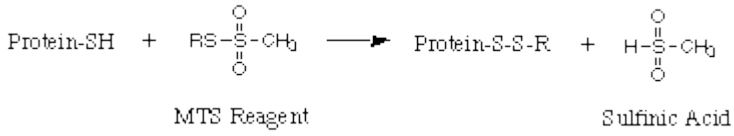
FT-AM376A
Storage: Stored at –20°C and protect from light

Introduction

Alkanethiosulfonate reagents, particularly MTS (methanethiosulfonate) reagents, were first developed by Dr. Arthur Karlin and colleagues as useful tools to probe the structures and functions of proteins, particularly membrane proteins such as ion channels. The reagents selectively and rapidly react with thiols (sulfhydryls) to form a disulfide bond and as a result are highly efficient labeling agents for cysteine residues in proteins.
The so-called SCAM method (substituted-cysteine accessibility method) employs a combination of chemical and genetic approaches. First, cysteine residues are systematically introduced at various positions in a protein via site-directed mutagenisis. Then the introduced cysteines are assessed on their reactivity and accesibility toward various MTS reagents. Determination is also made on the effect of the labeled cysteine on protein function. By using a series of MTS reagents differing in charge or size of the reagents, SCAM can yield information on the physical size and electrostatic potential of an ion channel, and on the membrane-sidedness and accessibility (buried or exposed) of a residue.

Our range of MTS reagents includes the commonly used small, charged and neutral MTS reagents for SCAM studies as well as fluorescent and biotinyl MTS derivatives. Fluorescent MTS reagents are useful for real-time studies of protein structure dynamics by measuring environment-dependent fluorescence, fluorescence lifetime or fluorescence resonance energy transfer (FRET).



Directions for use

Handling and Storage

Some methanethiosulfonates are hygroscopic and all hydrolyze in water, over a period of time, particularly in the presence of nucleophiles. They should be stored in a disiccator at -20°C and warmed up to room temperature before opening the vial. For maximum results, **solutions should be made up immediately prior to use** even though solutions in distilled water appear to be stable for hours at 4°C.

Instructions for use

MTS reagents decompose in buffer very quickly.
DMSO is a good solvent for the MTS reagents which are not water soluble (i.e. the non-charged MTS reagents).
MTSET is soluble in water or DMSO.

The MTS reagents can be employed in whole cell current measurements to identify changes in mutants from wild type behavior or in single channel recording.

The intrinsic reactivity of MTS reagents with thiols is quite high, on the order of 10^5 M^{-1} sec^{-1}. Similar rates can often be achieved with introduced cysteines in proteins, meaning that complete modification can be achieved using a few seconds of application and reagent concentrations in the 10-100 µM range (assuming a stoichiometric excess of reagent or continuous application of fresh reagents). Slower rates of modification may indicate that the introduced cysteine is not at the freely accessible surface of the protein, but is partially buried in a crevice or possibly in the pore of a channel protein. When modification is monitored by a functional measurement rather than by protein chemistry, a failure to see an effect of an MTS reagent may indicate either that the modification reaction did not occur or that even when modification does occur it produces no functional change in the assay used.

Sometimes an introduced cysteine may exhibit different modification rates depending on the conformational state of the protein. This phenomenon has allowed the MTS reagents to be used to analyze the nature of ion-channel gating motions.

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A change in conductance of a channel may be caused by a change in protein structure caused by modification of a cysteine at a remote site.

Of the sulfhydryl active reagents the MTS derivatives are, by far, the most rapidly reacting. Even so, their application is over a relatively long time relative to the time frame of protein motion. As a consequence the reagent may react with a minor channel conformation. Attempts to overcome the problem using very brief applications of reagents have been reported.

When used for determining membrane protein topology, it is important to consider the ability of MTS compounds to cross membranes. Yellen and his colleagues (Holmgren *et. al*., 1996) demonstrated that although MTSES and MTSET are membrane impermeant, MTSEA can modify membrane proteins from the "wrong side". The rate of wrong-sided or "trans" modification in excised membrane patches was about 30-fold slower than for right-sided application. Even the normally membrane impermeant MTSET could produce trans-membrane modification in patches that showed a transient electrical leak. The use of a thiol scavenger (such as 20 mM cysteine), on the opposite side of the membrane from where the MTS reagent is applied, is recommended to eliminate this "trans" modification.

MTSET is used for probing the structures and functions of the proteins. In conjunction with site-directed introduction of cysteins to proteins, a selected MTS reagent can add a charge (positive or negative depending on the MTS reagent) to the previously neutral cysteines. MTSET adds a positive charge to a thiol. At pH 7.5 and ambient temperature, MTSET hydrolyzes with a half-life of about 10 minutes. 1 mM MTSET can be routinely used and applied for 1 to 5 minutes.

References

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