

# **MTS reagents**

Positively charged reagent that reacts very rapidly and specifically with cysteine group. Useful tools for protein (transporters, receptors...) structure and activity studies

# **Description - Charged MTS reagents**

#### MTSEA 996180

2-Aminoethyl MethaneThioSulfonate Hydrobromide

#### CAS:16599-33-0; MW 236.16 (x)

Soluble in Ethanol, Methanol, Water

Half-life (pH7.0, 20°C): ca 12 min, Half-life (pH6.0, 20°C): ca 92 min, Half-life (pH7.0, 4°C): ca 116 min (Karlin 1998)

# MTSEA-biotin R57520

2-((biotinoyl)amino)ethyl MethaneThioSulfonate CAS:162758-04-5: **MW 381.52** 

Soluble in DMF, or DMSO at >10 mg/mL at 20°C. This reagent would fit inside a cylinder about 0.6nm in diameter and 1nm in length (Akabas 1992).

Also available as MTSEA-**lc**-Biotin, #AM3700 (MW607.7)

# MTSES AM3720

 $So dium\ (2-sulfonatoethyl) Methane Thio Sulfonate$ 

CAS:184644-83-5; MW 242.27 (J)

Soluble in DMF, DMSO, Hot Ethanol, Methanol, Water Half-life (pH7.0, 20°C): ca 370 min (Karlin 1998)

### MTSET U03510

(2-(trime thy lammonium) ethyl) Me thane Thio Sulfonate bromide

CAS:91774-25-3; MW 278.24 (J)

Half-life (pH7.0, 20°C): ca 11.2 min Half-life (pH6.0, 20°C): ca 55 min (Karlin 1998)

# MTSPA AM3731

 $3-Aminopropyl\,Methane Thio Sulfonate\,Hydrobromide$ 

CAS 92953-13-4; MW 250.15 (x)

Soluble in DMSO, eOH

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 Other MTS reagents :
 Charged MTS reagents | Neutral MTS reagents | Spin labelled MTS reagents |

 Fluorescent labelled reagents | Biotinylated MTS reagents | Photoaffinity reagents | MTS Crosslinkers



# 100mg 500mg 1g



Soluble in water, DMSO or DMF References



#### 100mg 250mg 500mg 1g



#### 100mg 500mg 1g





O H3SSCH2CH2CH2NH3<sup>+</sup>Br<sup>−</sup> O

References



# **Directions for use**

#### **Storage and Handling**

Some methanethiosulfonates are hygroscopic and all hydrolyse in water, over a period of time, particularly in the presence of nucleophiles. They should be stored in a disiccator at  $-20^{\circ}$ 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  $4^{\circ}$ C.

· DMSO is a good solvent for the MTS reagents which are not water soluble (i.e. the non-charged MTS reagents).

MTS reagents decompose in buffer very quickly (hydrolyses) more or less rapidly (see half-life in page 1).

#### Protocols

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Refer to the literature and following general information.

Routinely, one can use 2.5 mM MTSEA, 1 mM MTSET, or 10 mM MTSES, applied for 1 to 5 minutes. (MTSET is 2.5 times as reactive with small sulfhydryl compounds as is MTSEA, and 10 times as reactive as MTSES). <sup>(ref)</sup>.

# **General Information**

• MTS reagents are alkylthiosulfonates that react easily with cysteine residues stoichiometrically under mild conditions. This make them favourable when compared to traditional reagents (iodoacetates, maleimides, and organomercurials)<sup>(Kenvon 1977)</sup>. This is a specific and rapid process by which cysteine sulfhydryls are converted to a disulfhyde. The reaction pathway is potentially reversible upon the addition of thiols such as DTT (#UP28425).

Protein-SH	+	о RS-S-CH Ö	<b>&gt;</b>	Protein-S-S-R	+	о н-s-сң
		MTS Reagent			2	Sulfinic Acid

• MTS reagents allow useful probing of the structures and function of proteins. Their use combined to sitespecific mutagenesis has proved to be an extremely useful technique in the mapping of membrane proteins, in example for ion channels and transports proteins, as well as enzymes and receptors.

The mapping of membrane proteins has advanced considerably with the advent of cloned and expressed membrane proteins and the use of site-directed mutagenesis. A useful strategy is to introduce individual cysteine residues at various positions in a protein and to observe modifications in cell functions or detect introduced cysteins in cell.

- The chemical modification by a charged MTS reagent of cysteine residues (that have been introduced in a specific protein by mutagenesis) may produce a measurable change in the function of the ion channel/transport protein, which can be measured by electrical recording or isotope flux. Such data give valuable information concerning the time-course, state dependence and membrane-sidedness of the accessibility of the cysteine (Akabas 1992, Stauffer 1994). This is referred to as substituted-cysteine-accessibility method (SCAM) (Akabas 1994). The MTS reagents can be employed in whole cell current measurements to identify changes in mutants from wild type behaviour or in single channel recording.
- Biotinylated MTS reagents, such as MTSE R5752 can be easily detected with (strept) avidin reagents, i.e.:

Streptavidin, peroxidase conjugated UP395880 Streptavidin, phosphatase alkaline conjugated UP518490

For example one may test the surface accessibility on cells of membrane proteins that contain cysteins, and may have been introduced by SCAM method.



# More information - Advantages of MTS Reagents and other AlkylThioSulfonates

• Sulfhydryl active reagents have been used as blocking and labeling groups, reporter groups, cross-linking groups and affinity labeling groups for the chemical modification of peptides and proteins (Kenyon1977). **Classic reactive functionalities** are maleimides, iodoacetates, and organomercurials, which are in general slow to react, therefore require long reaction times and large excess of reagent. **Alkylthiosulfonates** are distinguished by their extremely rapid reactivity under the mild conditions necessary for successful electrophysiological recording experiments, their <u>high</u> <u>selectivity for cysteinyl</u> sulfhydryls, their ability to effect <u>quantitative and complete conversion</u> to the disulfide without applying a large excess of reagent, the general <u>reversibility</u> of the formation of disulfide bond upon the addition of thiols such a β-mercaptoethanol or dithiothreitol (Kenyon1977), and the wide range of functionality accommodated in the R group.

Even at mM concentration of proteins, stoichiometric sulfhydryl group modification may be achieved in solutions of either anhydrous organic or buffered aqueous and aqueous-organic solvents. Also, the sulfinic acid by-product of the reaction of a sulfhydryl with a methanethiosulfonate, decomposes rapidly to low-molecular-weight volatile products which do not, in general, affect the stability of the disulfide bond formed, or the activity of the enzyme <sup>(Bruice and Kenyon, 1982)</sup>.

The intrinsic reactivity of MTS reagents with thiols is quite high, on the order of  $10^5$  M-1 sec-1 <sup>(Staufferand Karlin, 1994)</sup>. Similar rates can often be achieved with introduced cysteines in proteins <sup>(Liu et al. 1996; Holmgren et al., 1996b)</sup>. Hence complete modification can be achieved using a few seconds of application and reagent concentrations in the  $10-100 \mu$ M 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.

-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 (Akabas et. al., 1992, 1994; Yang and Horn, 1995; Yang et al. 1996; Larsson et al., 1996; Liu et al., 1996).

• 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. 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 <sup>(Mindell et. al 1994)</sup>.

• MTS derivatives are, by far, the most rapidly reacting amongst the sulfhydryl active reagents. 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 <sup>(Lu and Miller, 1995)</sup>. Attempts to overcome the problem using very brief applications of reagents have been reported <sup>(Cheung and Akabas, 1996)</sup>.

• When used for determining membrane protein topology, it is important to consider the ability of MTS compounds to cross membranes. Although MTSES and MTSET are membrane impermeant, MTSEA can modify membrane proteins from the "wrong side" <sup>(Yellen andhis colleagues, in Holmgren et. al., 1996)</sup>. 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.





## **Charged MTS Reagents**

Arthur Karlin and his colleagues introduced three charged MTS reagents, 2-Aminoethyl methanethiosulfonate hydrobromide (MTSEA, Cat. # 996180), Sodium (2-sulfonatoethyl) methanethiosulfonate (MTSES, Cat. # AM3720), and [2-(Trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET, Cat. # U03510). These reagents were used in conjunction with site specific introduction of cysteines to study the structure and function of ion channel proteins (SCAM). Because these reagents introduce a positive or negative charge at the position of a previously neutral cysteine residue they frequently give a functional change in a channel protein that can be measured by electrical recording <sup>(Stauffer and Karlin, 1994; Akabas et al., 1992)</sup>.

SCAM and the charged MTS reagents have been successfully applied to the structural and functional elucidation of a number of ligand-gated ion channels, including muscle acetylcholine receptor <sup>(Akabas et al., 1992, 1994a, 1995)</sup>, neuronal acetylcholine receptor <sup>(Ramirez-Latore et al., 1996)</sup>, GABA receptor <sup>(Xuand Akabas, 1993, 1996)</sup>, NMDA glutamate receptor <sup>(Kuner et al., 1996)</sup>, and cyclic nucleotide gated channels <sup>(Sun et al., 1996)</sup>. This technique has also been applied to the cystic fibrosis transmembrane conductance regulator <sup>(Akabas et al., 1994b)</sup>, and to voltage-gated potassium <sup>(Pascual et al., 1995; Kürz et al., 1995)</sup> and sodium channels <sup>(Yang et al., 1996)</sup>. SCAM has also been used to map the ligand-binding domain of the seven-transmembrane-helices, G-protein-linked dopamine receptor <sup>(Javitchet al., 1995; Fu et al., 1996)</sup>.

The serotonin transporter belongs to a large family of integral membrane proteins responsible for terminating the action of neurotransmitters released from presynaptic neurons. Gary Rudnick and his colleagues used site-directed mutagenesis and MTS reagents to study this transporter <sup>(Humphreys et. al., 1994; J.-G. Chenet. al., 1997)</sup> and have identified the specific amino acid residues important for binding serotonin and cocaine and for conformational changes.

Ion channels are dynamic transmembrane proteins that undergo conformational changes when they open and close. Several physiologically important factors influence this gating process, including binding of agonists and changes of the transmembrane potential. However the way the channel protein transduces these signals into gating is largely unknown. Dick Horn and his colleagues have studied a particular voltage-dependent conformational change in sodium channels, which are responsible for the action potential in excitable cells. Using site-specific mutagenesis, they showed that the transmembrane potential affects the accessibility of the cysteine residues to the methanethiosulfonate reagents MTSES and MTSET (Yang and Horn, 1995; Yang, George and Hom, 1996).

Charged MTS Reagent	Short name	Structure	Cat.#
2-Aminoethyl methanethiosulfonate hydrobromide; CAS: 16599-33-0 ; MW:236.16 <sup>(M)</sup>	MTSEA-Bromide	H <sub>3</sub> C <sub>S</sub> S <sub>NH2</sub> · HBr	996180
2-Aminoethyl methanethiosulfonate hydrochloride; CAS:37597-96-9 ; MW:191.7 <sup>(M )</sup> Soluble in DMSO, Warm Ethanol, Methanol, Water <u>FT-U54991</u>	MTSEA-Chloride	H <sub>3</sub> C <sub>S</sub> S <sub>NH2</sub> · HCl	U54991
Methanesulfonothioic Acid S-(2-Aminoethyl) Ester-DBCO For click reactions with azide <u>FT-0C3640</u>	MTSEA-DBCO		0C3640
3-Aminopropyl methanethiosulfonate hydrobromide; CAS:92953-13-4; MW:250.18 <sup>(M)</sup> Soluble in DMSO, Methanol	MTSPA	0,0 H <sub>2</sub> N S S.Me	AM3731
2-Guanidinoethyl2-guanidinoethanethiosulfonate dihydrobromide; CAS:- ; MW:813.14 <sup>(M)</sup> Soluble in DMSO, Methanol, Water	-		RR5830 • 2HBr
Sodium (4-sulfonatobutyl)methanethiosulfonate; CAS:385398-78-7; MW:270.32 <sup>(M)</sup> Soluble in DMSO, Methanol, Water	MTSBS	0,0 0,0 Me <sup>-S</sup> S	RW3110
(2-sulfonatoethyl)methanethiosulfonate; CAS:1950-85-2; MW:233.29	MTSES		U03500

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	Short name	Structure	Cat.#
Charged MTS Reagent			
Sodium (5-sulfonatopentyl) methanethiosulfonate;	MTSPES	0,0	RW3120
CAS:385398-80-1 ; MW:284.35 <sup>(M)</sup> Soluble in DMF, DMSO, Methanol		H <sub>3</sub> C <sup>S</sup> S SO <sub>3</sub> Na	
Sodium (3-sulfonatopropyl) methanethiosulfonate;	MTSPS	H <sub>3</sub> C <sub>2</sub> S <sub>2</sub> S <sub>3</sub> Na <sup>+</sup>	RW3130
CAS:385398-83-4; MW:256.3 <sup>(M)</sup> Soluble in DMSO, Water		0 <sup>°</sup> 0	
6-(Triethylammonium) hexyl methanethiosulfonate bromide;	MTS-TEAH	Et <sub>3</sub> N+	RQ1890
CAS:386229-78-5; MW:376.43 <sup>(M)</sup> Soluble in DMSO, Methanol, Water		Br- O´`O	
3-(Triethylammonium)propyl methanthiosulfonate bromide;	MTS-PtrEA	Me	RW6000
CAS:219789-15-8; MW:334.35 <sup>(M)</sup> Soluble in DMSO, Methanol, Water		0,0	
1-Biotinylamino-3,6,9-trioxaundecane-11-ol	MTS-PEO3-OH		BV5950
CAS: [1322625-82-0; possible relative: 1217609-84-1; MW:419.54; (M) Soluble in Chloroform, Dichloromethane			
1-Biotinylamino-3,6,9-trioxaundecane-11-bromide;	MTS-PEO3-Bromide		RS7010
CAS: 1041766-91-9; MW:482.43; (K) Soluble in Chloroform, Methanol			
[2-(Trimethylammonium)ethyl] methanethiosulfonate bromide;	MTSET	Me	U03510
CAS:91774-25-3 ; MW:278.24 <sup>(M)</sup> Soluble in Methanol, Water		Me S N + · Br- 0 0 Me Me	
[2-(Trimethylammonium)ethyl] methanethiosulfonate bromide	MTSET <sup>14</sup> C <sub>2</sub>	H <sub>2</sub> H <sub>2</sub> Me	RW6570
$^{14}C_2$ ; CAS:- ; MW:282.22 <sup>(M)</sup>		Me_S_S14C14CN <sup>+</sup> · Br-	
Soluble in DMSO, Water			
$\label{eq:constraint} 2-(Trimethylammonium) ethyl toluenethiosulfonate bromide;$	TMA-ETS	CH <sub>3</sub>	RW6590
CAS:- ; MW:354.32 <sup>(M)</sup> Soluble in DMSO, Water		H <sub>3</sub> C-N <sup>+</sup> S <sub>S</sub> Br H <sub>3</sub> C-N <sup>+</sup> CH <sub>3</sub> O O	
(Trimethylammonium)methylmethanethiosulfonate bromide;	MTSMT		RQ1900
CAS:386229-81-8; MW:264.73 <sup>(M)</sup> Soluble in DMSO, Methanol			
3-(Trimethylammonium)propyl methanethiosulfonate bromide;	MTSPT	- H <sub>3</sub> C,	RW6650
CAS:220560-60-1 ; MW:292.26 $^{(\infty)}$ Soluble in DMSO, Metahnol, Water		Br H <sub>3</sub> C'+ SSCH <sub>3</sub>	
2-Carboxyethyl Methanethiosulfonate, Choline Ester Chloride		О, О О H <sub>3</sub> C, CH <sub>3</sub>	RT1510
Salt; CAS:- ; MW:305.84		$\begin{array}{ c c c c c }\hline 0,0&0&H_3C,CH_3\\ \hline H_3C,S,S&-&0&N^{+}CH_3\\\hline \end{array}$	• CI <sup>-</sup>
2-(Aminocarbonyl)ethyl methanethiosulfonate;	Cys-MTS	0,0	RQ0510
CAS:351422-29-2; MW:199.25 <sup>(X)</sup> Sparingly soluble in DMSO, Water		H <sub>3</sub> C <sup>S</sup> S <sup>C</sup> O <sub>2</sub> H NH <sub>2</sub>	
Ge, P., and Selvin, P.R.: Bioconjugate Chem., 14, 5, 870 (2003)			

 MTSL
 Inquire WZ781

 1-oxyl-2,2,5,5-tetramethyl-d3-pyrroline-3-methyl) methanethiosulfonate; MW 264.30
 MTS Kit (4 MTS reagents)

 MTS Kit (4 MTS reagents)
 Inquire WZ752

 Each kit contains 100mg of MTSEA, MMTS, MTSES, and MTSET.5x500mg kit is #WZ7510.

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P.5



#### **Neutral MTS Reagents**

Uncharged MTS reagents are also useful sulfhydryl active reagents for structure studies of known or unknown structure, particularly when used in conjunction with site-specific mutagenesis.

The MMTS #417311 reagent is a small reversible blocker of cysteines, facilitating the study of enzymes activation and other protein functions<sup>1]</sup>. Mass fingerprinting of peptides can be performed using MMTS, that modify thiol enzymes and redox-regulated proteins. For example, MMTS-treated forms of SF Ttc- $\alpha$  and GADPH by modified trypsin or Glu-C protease were analyzed by MALDI-TOF/TOF mass spectrometer equipped with a Smartbeam-II laser (Nd:YAG, 355 nm).<sup>[Makarov 2019]</sup>.

A study <sup>(Chahine et. al. 1997)</sup> showed that MTSBn reagent restored function in a channel that had been made inactivation-defective by the substitution of the phenylalanine at position 1486 with cysteine, supporting the theory that the phenyl group of the phenylalanine may play a crucial role in inactivation gate closure.

The thermal cis-trans isomerization of the carbamate MTSAC reagent was found (Foong et. al., 1997; Woolley et. al., 1995) to alter the flux of Cs+ ions through the gramicidin channel, detected as steps in single-channel recordings.

Neutral MTS Reagent	Short name	Structure	Cat.#
Allyl methanethiosulfonate CAS:14202-77-8; MW:152.241 <sup>(K)</sup>	Aliyi MTS	$\begin{bmatrix} 0 & 0 \\ H_3 C & S \\ S & S \end{bmatrix} C H_2$	RO8900
2-Amino-2-carboxyethyl methanethiosulfonate CAS:351422-28-1; MW:183.25	-		RQ0500
2-(4-Aminobenzoyloxy)ethyl methanethiosulfonate; CAS:- ; MW:183.25 <sup>(M)</sup>	-	H <sub>2</sub> N 0 0 0	
Benzocaine methanethiosulfonate CAS:212207-24-4 ; MW:275.34 <sup>(M)</sup> Soluble in Acetone, Chloroform, Ethanol, Methanol	-		Inquire
Benzyl methanethiosulfonate CAS:7559-62-8 ; MW:202.3 <sup>(M)</sup> Soluble in Acetone, Chloroform, Dichloromethane, Ethanol, Ether, Methanol	MTSBn	O, O S <sup>-S-</sup> CH <sub>3</sub>	RR1620
Butyl methanethiosulfonate CAS:52017-46-6; MW:168.28 <sup>(K)</sup> Soluble in Ethanol, Ethyl Acetate, Hexane	•	O, O H <sub>3</sub> C <sup>-S</sup> S <sup>-</sup> CH <sub>3</sub>	RQ4340
2-Carboxyethyl methanethiosulfonate; CAS:92953-12-3 ; MW:184.23 <sup>(M)</sup> Soluble in Acetone, Chloroform, Dichloromethane, Ethyl Acetate, Methanol	MTSCE	$\begin{bmatrix} O, O \\ H_3C^{-S} & CO_2H \end{bmatrix}$	CG2510
Decyl methanethiosulfonate CAS:190852-38-1 ; MW:252.44 <sup>(M)</sup> Water sensitive Soluble in Ethanol, Ethyl Acetate, Hexane	-	0,0 H <sub>3</sub> C <sup>-S'</sup> S <sup></sup> CH <sub>3</sub>	RP4020
Dodecyl methanethiosulfonate CAS:355803-77-9; MW:280.5 <sup>(X)</sup> Soluble in Dichloromethane	-	H <sub>3</sub> C <sub>S</sub> S O O	RQ0710
N-( $\beta$ -D-Glucopyranosyl)-N'-[(2-methanethiosulfonyl)ethyl]urea; CAS:- ; MW:360.41 <sup>(X)</sup> Soluble in Methanol	MTS-5-Glucose		RU5060
Hexadecyl methanethiosulfonate Hexane; CAS:7559-47-9 ; MW:336.6 <sup>(M</sup> Solubled in Chloroform, Dichloromethane, Ethyl Acetate Water sensitive		Me <sup>-S</sup> S	RR1610



Neutral MTS Reagent	Short name	Structure	Cat.#
2-Hydroxyethyl methanethiosulfonate; CAS:13700-08-8 ; MW:156.22 <sup>(K)</sup> Soluble in Dichloromethane, Ether, Ethyl Acetate, Methanol	MTSHE	Me <sup>SS</sup> S OH	RO8240
6-Hydroxyhexyl methanethiosulfonate; CAS:212261-98-8; MW:212.33	6-HH-MTS	H <sub>3</sub> C <sub>S</sub> S O O	RP5700
O-2-(Methanethiosulfonyl)ethyl N-[2-(N,N- dimethylamino)ethyl] carbamate, hydrochloride; CAS:185792-54-7; MW:306.83	MTS-AC	Me <sub>2</sub> N N O S'S Me · HCl	RP3710
3-Methanethiosulfonyl-N,N-dimethylpropionamide; CAS:359436-82-1; MW:211.3 $^{\scriptscriptstyle (X)}$	MTS-DMPA	H <sub>3</sub> C <sub>5</sub> S OOD OOD OCH <sub>3</sub>	RQ1000
Methoxypoly(ethylene glycol)-5000- succinamidoethyl methanethiosulfonate	MTS-PEG5000		Inquire
Methoxypoly(ethyleneglycol)20 Amidopropionyl Methanethiosulfonate	MTS-mPEG20		
Methyl methanethiolsulfonate; CAS:2949-92-0 ; MW:126.20 <sup>(M)</sup>	MMTS	H <sub>3</sub> C <sub>S</sub> S <sub>CH3</sub>	417311
Propyl methanethiosulfonate; CAS:24387-69-7 ; MW:154.25	-	Me <sup>'S</sup> s Me	RP7230
Pentyl methanethiosulfonate; CAS 4212-65-0; MW:182.31	-	Me <sup>S</sup> S Me	RQ2800
Octyl methanethiosulfonate; CAS:7559-45-7 ; MW:224.38	-		RR1600
Pyridinedithioethylamine, hydrochloride	PDA		Inquire

# Spin Labeled MTS Reagents (MTSL)

FT 006180 (MTS reagants)

The spin labeled derivative of MTSL, proxyl-MTS (1-Oxyl-2,2,5,5-tetramethyl-Æ3-pyrrolin-3-yl)methyl methanethiosulfonate) has been described (Berliner et. al., 1982): it exhibits high sulfhydryl selectivity and reactivity. The side-chain has a relatively small molar volume, and the EPR spectrum, which measures the accessibility to collision with paramagnetic species in solution (an indication of its solvent accessibility) and the motion of the spin-labeled side-chain, is exquisitely sensitive to structural changes. Site directed spin labeling (SDSL) and analysis of the electron paramagnetic resonance of spin labeled proteins can be used to map the topography of a membrane protein, to determine secondary structure, measure the distance between two sites bearing a spin label, and identify sites of tertiary interaction. The ability to time-resolve these structural features makes SDSL a powerful approach for exploring the evolution of structure on the millisecond time-scale. Future applications are anticipated to include the study of protein folding both in solution and in chaperone-mediated systems (Hubbell et al. 1996, Hubbell and Altenbach 1994, Hubbell and Altenbach in : Membrane Protein Structure: Experimental Approaches, 1994).

MTSL was used in the study of T4 lysozyme <sup>(Mchaourabet. al., 1996 - a key paper for interpreting the MTSL spectral lineshape)</sup>. The quantitative analysis of spin-spin interactions between nitroxide pairs revealed an 8 Å relative movement upon substrate binding <sup>(Mchaourabet. al., 1997)</sup>. MTSL was also used in studies of:

1. the interaction of the toxin colicin E1 with membranes (Shinet. al. 1993);

2. structure, and structural changes of rhodopsin (Farrens et. al., 1996), and bacteriorhodopsin during the photocycle (Altenbach et. al. 1990; Steinhoff et. al. 1994);

3. the diphtheria toxin transmembrane domain (Oh et. al., 1996).





Spin Labeled MTS Reagent	Short name	Structure	Cat.#
(1-Oxyl-2,2,5,5-tetramethylpyrrolidin-3-yl)methyl methanethiosulfonate; CAS:201403-46-5; MW:266.4 <sup>(M)</sup>	proxyl-MTS	$\begin{array}{c} O, O\\ S, S\\ H_3C\\ H_3C\\ O\\ O\\$	RV9770
(1-Oxyl-2,2,5,5-tetramethyl-Æ3-pyrrolin-3-yl)methyl methanethiosulfonate; CAS:81213-52-7; MW:264.39 <sup>(M)</sup>	MTSL		BU2956
(1-Oxyl-2,2,5,5-tetramethyl-Æ3pyrrolin-3- yl)methyl methanethiosulfonate-d <sub>15</sub> ; CAS:384342-57-8; MW:239.7	MTSL-D <sub>15</sub>	$\begin{array}{c} D & O, O \\ D & D & S \\ \hline D_3C & V & CD_3 \\ \hline D_3C & N & CD_3 \\ \hline O & O \end{array}$	RV9790
(1-Oxyl-2,2,5,5-tetramethyl-Æ3pyrrolin-3- yl)methyl methanethiosulfonate- <sup>15</sup> N-d <sub>15</sub> ; CAS - ; MW:280.49	MTSL- <sup>15</sup> N-D <sub>15</sub>	$\begin{array}{c} D_{2} & O_{2} \\ D_{3}C & C_{-S} & S^{-}CH_{3} \\ D_{3}C & CD_{3} \\ D_{3}C & CD_{3} \\ 0 \end{array}$	RV9800
(1-Oxyl-2,2,5,5-tetramethylpyrrolin-3- yl)carbamidoethylmethanethiosulfonate; CAS:384342-59-0; MW:321.44	MTS-4-Oxyl		RV9810

### **Fluorescent MTS Reagents**

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Fluorescent MTS- reagents may find application in the real-time monitoring of conformational changes, since fluorophores coupled to introduced cysteines can change their fluorescence during a conformational change (Manuzzu et al., 1996). Fluorescence lifetime may also yield information regarding distances and molecular motion in a protein molecule.

Fluorescent Labeled MTS Reagent	Short name	Structure	Cat.#
N-[4-(Aminosulfonyl)-2,1,3-benzoxadiazol-7-yl]-2- aminoethyl methanethiosulfonate; CAS:35200-01-2; MW:352.41	ABD-MTS		FP-CP7020
Dansylamidoethyl methanethiosulfonate [2-(5- Dimethylaminonaphth-1-yl sulfonamido)ethyl methanethiosulfonate]; CAS:355115-41-2; MW:388.53	MTS-Dansyl	Cost Stores	FP-CP7030
(N-Dansyl)biocytinamidoethyl methanethiosulfonate; CAS:255115-41-2; MW:743	MTS-DB		FP-RT4840
N-(methanethiosulfonylethylcarboxamidoethyl)-5- naphthylamine-1- sulfonic acid, sodium salt; CAS 359436-83-2; MW:454.51	MTS-1,5-EDANS- Carboxyethyl		FP-RV1080
2-[(5-Fluoresceinyl)aminocarbonyl]ethyl methanethiosulfonate; CAS:-]: MW:513.55	MTS-4-Fluorescein	HO CO CH	FP-R59301



Fluorescent Labeled MTS Reagent	Short name	Structure	Cat.#
Cyanine 3 Monofunctional MTSEA Dye, Potassium Salt; MW:806.09; (L) Appearance: Red Solid Melting Point: 306-307°C (dec.) Solubility: DMF, DMSO, Methanol, Water	Cy3-MTSEA	O <sub>3</sub> S H <sub>3</sub> C CH <sub>3</sub> H <sub>3</sub> C CH <sub>3</sub> SO <sub>3</sub> Y N <sup>+</sup> CH <sub>3</sub> CH <sub>3</sub>	FP-CP2190
Cyanine 5 Bisfunctional MTSEA Dye, Potassium Salt; MW:1055.44 Appearance: Red Solid Melting Point: >240°C (dec.) Solubility: DMF, DMSO, Methanol, Water	Cy5-diMTSEA	O3S Me Me Me Me SO3K	FP-RT4230
N-[2-Methanethiosulfonylethyl]-7-methoxycoumarin- 4-acetamide; CAS:887406-79-3; MW:371.43	MTS-EMCA		FP-RR5040
2-(Pyren-1-ylaminocarbonyl)ethyl methanethiosulfonate; CAS:384342-67-7; MW:383.49	Pyrene-ACE-MTS	N S'S'Me	FP-RQ1770
1-Pyrenylmethyl methanethiosulfonate; CAS:384342-65-8; MW:326.44	Pyrene-1-MTS	O, O S <sup>-S'</sup> Me	FP-RQ1780
2-[(3-Pyrenylpropyl)carboxamido]ethyl methanethiosulfonate; CAS:384342-66-9; MW:425.56	Pyrene-7-MTS	H O O	FP-RQ1790
(2-Pyridyl)dithiobimane; CAS:385398-64-1; MW:333.43	PDT-Bimane		RQ1800
Sulfo-rhodamine methanethiosulfonate; CAS:386229-71-6; MW:695.88	MTSR	$\begin{array}{c} H_{3}C & CH_{3} \\ H_{3}C & N & CH_{3} \\ & & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & &$	FP-RW3650
SulfoRhodamine101-2-Sulfonamidoethyl methanethiosulfonate; CAS:- ; MW:743.94 <sup>(M)O</sup> Soluble in Chloroform, DMSO ; A/E:582/600	SR101-MTSEA	$\begin{array}{c} \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	FP-BJ1970



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### **Biotin Labeled MTS Reagents**

Spin Labeled MTS Reagent	Short name	Structure	Cat.#
N-Biotinoylaminoethyl methanethiosulfonate CAS:162758-04-5; MW:381.54	MTSEA-Biotin	HN NH O S S S CH <sub>3</sub>	RP1620
N-Biotinoylcaproylaminoethyl methanethiosulfonate CAS:353754-95-7; MW:494.7	MTSEA-X-Biotin		AM36901 RQ0600
Biotin-[2-(2-pyridyldithio)ethylamide] CAS:112247-65-1; MW:412.6	PDTE-Biotin		RO4700
(N-Dansyl)biocytinamidoethyl methanethiosulfonate CAS:- ; MW:743	MTS-DB		RT4845
1-Biotinylamino-3,6,9-trioxaundecane-11-yl- methanethiosulfonate CAS:1217838-20-4 (relative stereo) ; MW:513.69; (K) Soluble de Chloroform, Dichloromethane, Methanol	MTS-PEO₃-Biotin		RZ7020

# **Photoaffinity Reagents**

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Photoreactive MTS Reagent	Short name	Structure	Cat.#
S-[2-(4-Azidosalicylamido)ethylthio]-2-thiopyridine CAS:164575-82-0; MW:347.42	AET, ASAETP	H H H H H H H H H H H H H H H H H H H	RP1750
4-Azido-2,3,5,6-tetrafluorobenzamidocysteine methanethiosulfonate CAS:35200-06-7; MW:416.33	ATFBC-MTS		RQ0520
Benzophenone-4-carboxamidocysteine methanethiosulfonate CAS:317821-69-5 ; MW:407.47	BPCAC-MTS	O ÇO <sub>2</sub> H N S S.Me O O O	RS3780
S-[2-(Iodo-4-azidosalicylamido)ethylthio]-2- thiopyridine CAS:175093-14-8; MW:599.21	IAET		RP2670
4-[[5-Oxo-5-(phenylmethoxy)pentyl] [(phenylmethoxy)carbonyl]amino]-1- piperidinecarboxylic Acid t-Butyl Ester CAS:181629-57-2; MW:524.64   Colourless Liquid   Soluble in Dichloromethane, Diethyl Ether, Methanol			RP3100



#### **MTS Crosslinkers**

MTS crosslinkers, which can be used to crosslink cysteines will find application in the topographical mapping of proteins. The use of different length linkers can assist in the determination of the distance between two cysteine residues. Crosslinkers might also be used to stabilise protein conformation.

Crosslinker MTS Reagent	Short name	Structure	Cat.#
1,1-Methanediyl bismethanethiosulfonate CAS:22418-52-6; MW:236.35 Moisture sensitive (M) Appearance: White to Off-White Solid Melting Point: 77-78°C Solubility: Acetone, Dichlormethane, DMF, Ethyl Acetate	MTS-1-MTS	0,0 0,0 H₃C <sup>∕S</sup> ∕S∕S∕S,CH₃	RP6570
1,2-Ethanediyl bismethanethiosulfonate CAS:55-95-8 ; MW:250.38 Moisture and temperature sensitive (M) Appearance: White to Off-White Solid Melting Point: 116-118° C Solubility: Dichloromethane, DMF, DMSO	MTS-2-MTS	Me <sup>-,S</sup> 's <sup>-,Me</sup> O'O	RQ5480
1,3-Propanediyl bismethanethiosulfonate CAS:55-96-9 ; MW:264.41 Moisture and temperature sensitive (M) Appearance: White to Off-White Solid Melting Point: C Solubility: Dichloromethane, DMF, DMSO	MTS-3-MTS	Me_s_S_SS_Me O´O O´O	RQ5490
1,4-Butanediyl bismethanethiosulfonate CAS:55-99-2 ; MW:278.43 Moisture and temperature sensitive (M) Appearance: White to Off-White Solid Melting Point: 84-86 C Solubility: Dichloromethane, DMF, DMSO	MTS-4-MTS	H <sub>3</sub> C <sub>S</sub> S 0 0 0	RQ5510
1,5-Pentanediyl bismethanethiosulfonate CAS:56-00-8 ; MW:292.46 Moisture and temperature sensitive (M) Appearance: White Solid Melting Point: 71-72°C Solubility: Dichloromethane, DMF, DMSO	MTS-5-MTS	0,0 0,0 Me <sup>2</sup> S <sup>2</sup> S <sup>2</sup> Me	RQ5520
1,6-Hexanediyl bismethanethiosulfonate CAS:56-01-9 ; MW:306.49 Moisture and temperature sensitive (M) Appearance: Off-White Solid Melting Point: C Solubility: Dichloromethane, DMF, DMSO	MTS-6-MTS	0,0 Me <sup>-S</sup> S-S-Me	RQ5530
3,6-Dioxaoctane-1,8-diyl bismethanethiosulfonate CAS:212262-04-9; MW:338.49 Moisture and temperature sensitive (M) Appearance: White to Off-White Solid Solubility: Dichloromethane, DMF, DMSO	MTS-8-PEO <sub>2</sub> -MTS	H <sub>3</sub> C <sup>·S·</sup> S <sup>·O</sup> <sup>O</sup> <sup>O</sup> <sup>S</sup> S <sup>·CH<sub>3</sub></sup>	U95040
3,6,9-Trioxaundecane-1,11-diyl bismethanethiosulfonate; CAS:212262-02-7; MW:382.54 Moisture sensitive (M) Appearance: White to Off-White Solid	MTS-11-PEO <sub>3</sub> -MTS	0,0 H <sub>3</sub> C <sup>,S</sup> ,S, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,	RP5710
3,6,9,12-Tetraoxatetradecane-1,14-diyl bismethanethiosulfonate CAS:212262-08-3; MW:426.59 Moisture sensitive (M) Appearance: White to Off-White Solid	MTS-14-PEO4-MTS	Me <sup>xS</sup> s <sup>x</sup> s <sup>2</sup> , 0, 0, 0, 0, 5, 5, Me	RP5720

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Short name	Structure	Cat.#
MTS-17-PEO₅-MTS	$H_{3} C - S_{0_{2}}^{S} \sim 0^{0} \sim 0^{0} \sim 0^{0} \sim 0^{0} \sim S_{0_{2}}^{S}$	<sub>CH3</sub> U95050
MTS-3-NHS	O O	RQ1810
	O_N_S_S_O_N_	
	H <sub>3</sub> C <sup>-S</sup> O O	
MTS-PF		BJ3200
MTS-Resin		BJ3210
	MTS-17-PEO5-MTS	$MTS-17-PEO_{5}-MTS$ $H_{0}-S_{2}^{*}S_{0}-\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}\sqrt{0}$

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Other references on inquire +





# **Other Information**

### **Related products and documents:**

\* <u>Associated products</u> :

DTNB #UP01566H (detection and quantitation of sulfhydryls)

Other thiol-specific labeling reagents, i.e. Maleimide-Biotins, Maleimide-FluoProbes, ...

thiol-specific crosslinkers, i.e. MAL-MAL, MAL-NHS

(strept)avidin reagents (conjugates SAV-HRP #UP395880, and SAV-Alcaline Phosphatase #UP518490) See Product hightlights, BioSciences Innovations catalogue and e-search tool.

Catalog size quantities and prices may be found at http://www.interchim.com.

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