EIFE SCIENCE



Bioconjugation Reagents



Bioconjugation is the formation of complexes by chemically bonding functional molecules to biomolecules such as DNA, RNA, proteins, lipids and sugars under mild conditions. The bioconjugated complexes are used to develop new methods, for example in drug discovery, ligand binding assays, disease diagnosis, and high-throughput screening. There have been many recent reports of the chemical modification of biomolecules with non-natural bioorthogonal functional groups such as azide.

Biotinylation Reagents

The avidin-biotin system is widely used for bioanalysis and bioassays including flow cytometry, ELISA, immunohistochemical staining, western blotting and others. Biotin labeling (biotinylation) is also commonly used for conjugating proteins, especially antibodies, and other various molecules. Biotinylation is one of the most essential methods in the field of immunoassay where antigens are detected using antibodies. Streptavidin is a protein from the avidin family having extraordinarily high affinity for biotin, in fact, the interaction of biotin with streptavidin is among the strongest non-covalent affinities known in nature. In order to detect the biotinylated substance, modification of streptavidin with fluorescent label or enzyme is required. The biotinylated substance and the labeled-streptavidin are used in various assays based on the avidin-biotin system.







*G0597 is the successor to Anti-Rabbit IgG Biotin Conjugate (Product Number: G0389). Please use G0597 alternatively if you have used G0389.

Crosslinkers for Copper-free Click Chemistry

Click reaction to azides proceeds without copper(I) species because these reagents have a strained structure with cyclooctyne.





Phenylazides

Phenylazide generates a nitrene by UV irradiation (<300 nm). It is noted that azido groups tend to have less harmuful effect on target analyte. Activation of the nitrene requires a shorter wavelength of UV light, and potential protein denaturation during long-period irradiation should be taken into consideration.



4-Azido-2,3,5,6-tetrafluorobenzoic Acid 4-Azido-2,3,5,6-tetrafluorobenzoic Acid *N*-Succinimidyl Ester 5-Azido-2-nitrobenzoic Acid *N*-Succinimidyl Ester Bis[2-(4-azidosalicylamido)ethyl] Disulfide 1g [A2674] 200mg / 1g [S0952] 10mg [S0860] 10mg [B3790]





R = CO-NHS [50863] R = maleimide [M3259] Benzophenone excited by UV irradiation (near 360 nm) to induce hydrogen abstraction from target molecules. The reaction efficiency remains high despite this due to the reverseability of the excited state. Additionally, photoexcited benzophenone is not water-reactive.

4-Benzoylbenzoic Acid N-Succinimidyl Ester New 4-(N-Maleimido)benzophenone 200mg / 1g [S0863] 50mg / 250mg [M3259]

PEGylation Reagents

Applicable to the preparation of PEGylated antibodies, antibody-drug conjugates, etc.

Select	tion Guide R Target Group Reactive Gr	oup (PEG) _n		
Target Group PEGylation Reagents				
Amino	NHS Ester – (PEG) _n $\left(\begin{array}{c} & & \\$	n=425mg [M2186]n=825mg [M2187]n=1225mg [M2188]		
Carboxyl	Amino Group — (PEG) _n H_2N $(O)_n^{CH_3}$	n=4 100mg [M2501] n=8 50mg / 250mg [O0457] n=24 25mg / 100mg [M3048]		
Thiol	Maleimide Group – (PEG) _n $\begin{cases} \downarrow & \downarrow \\ & \downarrow & \downarrow \\ & & \downarrow & \downarrow \\ & & & 0 \end{cases} \overset{H}{\underset{O}{I}{\overset{H}{\underset{O}{\overset{H}{\underset{O}{\overset{H}{\underset{O}{\overset{H}{\underset{O}{I}{\overset{H}{\underset{O}{\overset{H}{\underset{O}{\overset{H}{I}{I}}{I}}}}}}}}}}}}}}}}}}}}}}}}}$	n=12 25mg [M3051] n=24 25mg [M3052]		
	Disulfide Group – (PEG) _n $(CH_2)_4$ $(CH_2)_4$ $(CH_2)_4$	n=4 100mg [T3199]		
Azido	Alkynyl Group – (PEG) _n ^{HC} Sc_o(o) ^{CH} 3	n=4 25mg/100mg [P2249]		
Alkyne / Cyclooctyne	Azido Group — (PEG) _n $N_3 \left(\begin{array}{c} & \\ & \\ & \\ & \\ & \end{array} \right)_n^{CH_3}$	n=4 25mg / 100mg [A2728] n=8 25mg / 100mg [A2727] n=12 25mg [M3049] n=24 25mg / 100mg [M3050]		
Amino, etc.	Bromo Group – (PEG) _n Br $(\bigcirc)_n^{CH_3}$	n=2 5g / 25g [B4736] n=3 5g / 25g [D3831] n=4 5g / 25g [T2634]		
Other	Hydroxy Group — (PEG) _n $HO(-)_{n}^{CH_3}$	n=2 25mL/500mL [M0537] n=3 25mL/500mL [T0709] n=4 5g/25g [T1372] n=5 1g/5g [P1159] n=6 1g/5g/25g [H0808] n=7 1g/5g [H1046] n=8 1g/5g [O0296] n=9 500mg/1g [N0699] n=12 100mg/1g [D2904]		

For Laboratory Use, Research Purposes Only.



Please inquire for pricing and availability of listed products to our local sales representatives.

Bifunctional Linkers				
selection gui	ide R Tar Gro	get Active up X Group X' (PEG)n Group	Y' Target Group Y R'	
arget Group X	Target Group Y	Linkers		
		NHS Ester-(Spacer)-NHS Ester	[D1662][D3895][D4019]	
Amino	Amino	NHS Ester-(Disulfide)-NHS Ester	[D2473]	
		Carboxyl Group-(Disulfide)-Carboxyl Group Aldehyde Group-(Spacer)-Aldehyde Group	[D0945][D0947][D1757][D3670] [G0067][G0068]	
		Imide Ester-(Spacer)-Imide Ester	[A0806][P0892][S0246]	
		Fluorobenzene-(Spacer)-Fluorobenzene	[D1649][D0536]	
	Carboxyl	Carboxyl Group-(Spacer)-Amino Group	[G0099][A0180][A0282]	
		NHS Ester-(Spacer)-Boc Amino Group	[A0663][A0312][A0311][A0932] [B5684]	
			[S0427][S0399][S0428][S0882]	
		NHS Ester-(Spacer)-Maleimide Group	[\$0853][\$0883][\$0398][\$0861] [\$0881]	
	Thiol	Carboxyl Group-(Spacer)-Maleimide Group	[M1962][M2337][M2338][M3143]	
	THIO	Carboxyl Group-(Spacer)-Thiol Group	[M0052] [S0431][S0859][S0819]	
		NHS Ester-(Spacer)-Protected Thiol Group Carboxyl Group-(Spacer)-Disulfide	[50431][50859][50819] [L0058]	
	Azido	Carboxyl Group-(Spacer)-Alkyne/Cyclooctyne	[P0497][H0882][U0054][P2341]	
	Alkyne / Cyclooctyne	Carboxyl Group-(Spacer)-Azide Group	[A2729]	
	Other	NHS Ester-(Spacer)-Acrylic Group	[S0814][S0812]	
		NHS Ester-(Spacer)-Other Group	[S0852][S0844][S0893]	
	Thiol	Amino Group-(Spacer)-Maleimide Group	[A2436] [A0648]	
Carboxyl	Azido	Amino Group-(Spacer)-Thiol Group Amino Group-(Spacer)-Alkyne/Cyclooctyne	[P0911][A2763]	
-	Alkyne / Cyclooctyne	Amino Group-(Spacer)-Azide Group	[A2738]	
Aldehyde	Aldehyde	Hydrazide Group-(Spacer)-Hydrazide Group	[C0803][O0083][S0482] [A0170][A0746][S0224][D2342]	
	Thiol	Hydrazide Group-(Spacer)-Maleimide Group	[M2703][M2735]	
Think	Thiol	Maleimide Group-(Spacer)-Maleimide Group [B3805][E0482][B1787] Maleimide Group-(Disulfide)-Maleimide Group [B5699]		
Thiol	Azido	Maleimide Group-(Disdinde)-Maleimide Group	[D4739][P2139]	
	AZIUU	Alkyne-(Spacer)-Hydroxy Group	[P0536][B0799][P0817][H0687]	
Azide	Other	Cyclooctyne-(Spacer)-Hydroxy Group	[H1474][O0445][D3710][U0055] [B5467]	
) 0		$O = C - (CH_2)_2 S - S(CH_2)_2 - C - O - N$	
5g / 25g [D1662] n=6 1g / 5g [D3895] 200mg / 1g / 5g [D2473] n=8 1g / 5g [D4019] 0				
$\begin{array}{c} O & O \\ HO-C-(CH_2)_n S-S(CH_2)_n -C-OH \\ HO-C-(CH_2)_n -C-OH \\ HO-C-(C$				
n=1 25g [D0945] H H				
n=2 25g / 100g / 500g [D0947] 0 n=3 (>95.0%) 5g / 25g [D1757] 25mL / 500mL [G0067] 1g / 5g [B5684] n=3 (>99.0%) 1g [D3670] 25mL / 500mL [G0068] 1g / 5g [B5684]				
F NO ₂ F NO ₂ g / 25g [D1649]	O₂N F	$\begin{array}{c}F \\ N & N \\ IO_2 & CH_3O-C - (CH_2)_n - C - OCH_3 \\ & \cdot 2HCI \\ n=3 \\ n=4 & 5g / 25g [A0806] \\ n=5 \\ n=5 & 5g / 25g [P0892] \\ n=6 & 5g / 25g [S0246] \end{array}$	100mg / 1g / 5g / 25g [<mark>S0399</mark> 100mg / 1g / 5g / 25g [<mark>S0428</mark>	

Bioconjugation Reagents



Protein-Oligosaccharide Conjugates

TCI offers carbohydrate-conjugated human serum albumin (HSA) which is manufactured using high-purity synthesized carbohydrates. Several sugar-conjugates are available, and it is also possible to manufacture the sugar-conjugates according to customer specifications. For more details on the products and contracts, please contact us.



1set [B6096]

1set [B6097]

Pre-Weighed Bioconjugation Reagents

for Biotin Conjugation

Biotin-LC-LC-NHS (2mg×5)

Biotin-PEG₂-NHS (2mg×5)

Applications

Preparation :

Use of a 10 mM biotinylation solution is recommended. In order to efficiently biotinylate a sample, biotinylation solution should be used at a 15-fold molar excess over the amount of amine-containing protein. Make sure to calculate the 10 mM biotinylation solution amount (see example below).

Calculate : A µL of 10 mM biotinylation solution for biotinylation 2 mg IgG (150,000 M.W.)

- 2 [mg lgG] x 10⁻³ [g/mg] x 1/150,000 [mol/g] x 15 [fold]
- = A [μ L of 10 mM biotinylation solution] x 10⁻⁶ [L/ μ L] x 10 [mmol/L] x 10⁻³ [mol/mmol]

A = 20 [μ L of 10 mM biotinylation solution]

Direction for Use :

- 1. Bring each product to room temperature.
- 2. Dissolve 2 mg of Biotin-LC-LC-NHS [B6096] in 350 μL of DMSO or DMF or 2 mg of Biotin-PEG₂-NHS [B6097] in 400 μL of PBS to prepare a 10 mM biotinylation solution.
- 3. Dissolve the sample (1-10 mg/mL) in an appropriate buffer such as PBS. Do not use buffers including amines (such as Tris).
- Add A μL of 10 mM biotinylation solution to the sample solution and incubate the mixed solution for 30 min at room temperature.
- 5. Remove unreacted and hydrolyzed reagent using desalting column or dialysis methods.

for Protein Conjugation via Thiol Groups

Bovine Serum Albumin Maleimide Conjugate (1mg×3) Horseradish Peroxidase Maleimide Conjugate (0.5mg×3) Streptavidin Maleimide Conjugate (0.5mg×1)

1set [B5944] 1set [H1621] 1set [T3531]

*B5944 is unavailable in the U.S. and China. H1621 and T3531 are also unavailable in China.

Application : HRP-labelling of an antibody with H1621

In case of antibodies without free thiol (SH, sulfhydryl) groups, disulfide moieties in proteins can be reduced by a reductant such as DTT [D3647] or 2-MEA [A0296] to reveal free thiols.

Furthermore, thiol group can be introduced to primary amines by adding SATA [S0431], SATP [S0859] or 2-Iminothiolane.



Example protocol for antibody conjugation starts from a reduction of native disulfide bonds in the Goat Anti-Mouse IgG, followed by labeling with the HRP using H1621. For more information, see the product detail page of H1621 on TCI website.

Protocol

- 1) Add DTT to a final concentration equal to 3 mole equivalents per mole equivalent of antibody present.
- 2) Incubate for 90 minutes at 37 °C.
- 3) Purify the reduced IgG by gel filtration or ultrafiltration, dialysis.
- 4) Add equal amount of H1621 (by weight) to a purified antibody and Incubate for 2 hours at room temperature (25 °C).

Functional Group Forming Agents and Condensing Agents

Thiol Group Formation (Disulfide Reduction) Reagents



Ordering and Customer Service

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