

Covalent coupling kits compatibility sheet

Buffer additives The conjugation of ligands with all available coupling kits can be performed with many different additives. The following list shows all tested additives. Please note that other additives, which are not listed here may successfully be used for conjugation.

	Up to	Suitability Amine Coupling	Suitability Thiol Coupling
EDTA	1 mM	● ○ ○	● ○ ○
DTT*	1 mM	● ○ ○	○ ○ ●
TCEP	1 mM	● ○ ○	● ○ ○
Tris**	—	○ ○ ●	● ○ ○
DMSO	2 %	● ○ ○	● ○ ○
ATP	0.5 mM	● ○ ○	● ○ ○
MgCl ₂	2.5 mM	● ○ ○	● ○ ○
Glycine**	—	○ ○ ●	○ ● ○
Mannitol	8 %	● ○ ○	● ○ ○
Glycerol	10 %	● ○ ○	● ○ ○
Trehalose	8 %	● ○ ○	● ○ ○
Histidin**	30 mM	○ ○ ●	○ ● ○
Acetonitrile***	50 %	● ○ ○	● ○ ○
Trifluoroacetic acid	0.1 %	● ○ ○	● ○ ○

* thiol-based reducing agents

** contains primary amines

*** caution, may harm the ligand

Suitability

● ○ ○	> 85 % yield
○ ○ ●	> 30 % yield
○ ○ ●	0-30 % yield or containing free thiols / primary amines

**Crosslinker
(solid –
undissolved)**

Please make sure to store the crosslinker at -20°C.

Stability: The crosslinker is even stable for 3 days when stored at room temperature inadvertently.

pH / pI

The pH value for the conjugation buffer may range from **pH 5.0** to **pH 8.0**, depending on the ligand characteristics.

When performing a conjugation of proteins with a pI of < 6, please note that using a buffer with lower pH may result in a better yield of conjugate.

	Composition
Phosphate-Citrate Buffer pH 5.0	50 mM buffer salt, 150 mM NaCl, pH x.x
Buffer M pH 6.5 (BU-M-150-1)	
Buffer A pH 7.2 (BU-P-150-10)	
Buffer C pH 8.0 (BU-C-150-1)	

Salt concentration

For standard conjugations 50 mM buffer salt and **150 mM NaCl** (monovalent salt) are used.

When performing conjugation of strongly charged ligands, make sure that the concentration of NaCl is sufficiently high (**up to 400 mM NaCl** is recommended). Otherwise, precipitation of DNA may occur.

The shielding effect of monovalent sodium cations leads to DNA stabilization through neutralization of the negative charge on the sugar phosphate backbone.

Important notes

Avoid using partially purified protein samples or protein samples containing carriers (e.g., BSA).

For amine coupling kits (HK-NHS-X):

- To get highest reaction yields, the ligand should be dissolved in Buffer C. Buffer exchange is recommended prior to conjugation process.

For thiol coupling kits (HK-MAL-X):

- To get highest reaction yields, the ligand should be dissolved in Buffer A. Buffer exchange is recommended prior to conjugation process.

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