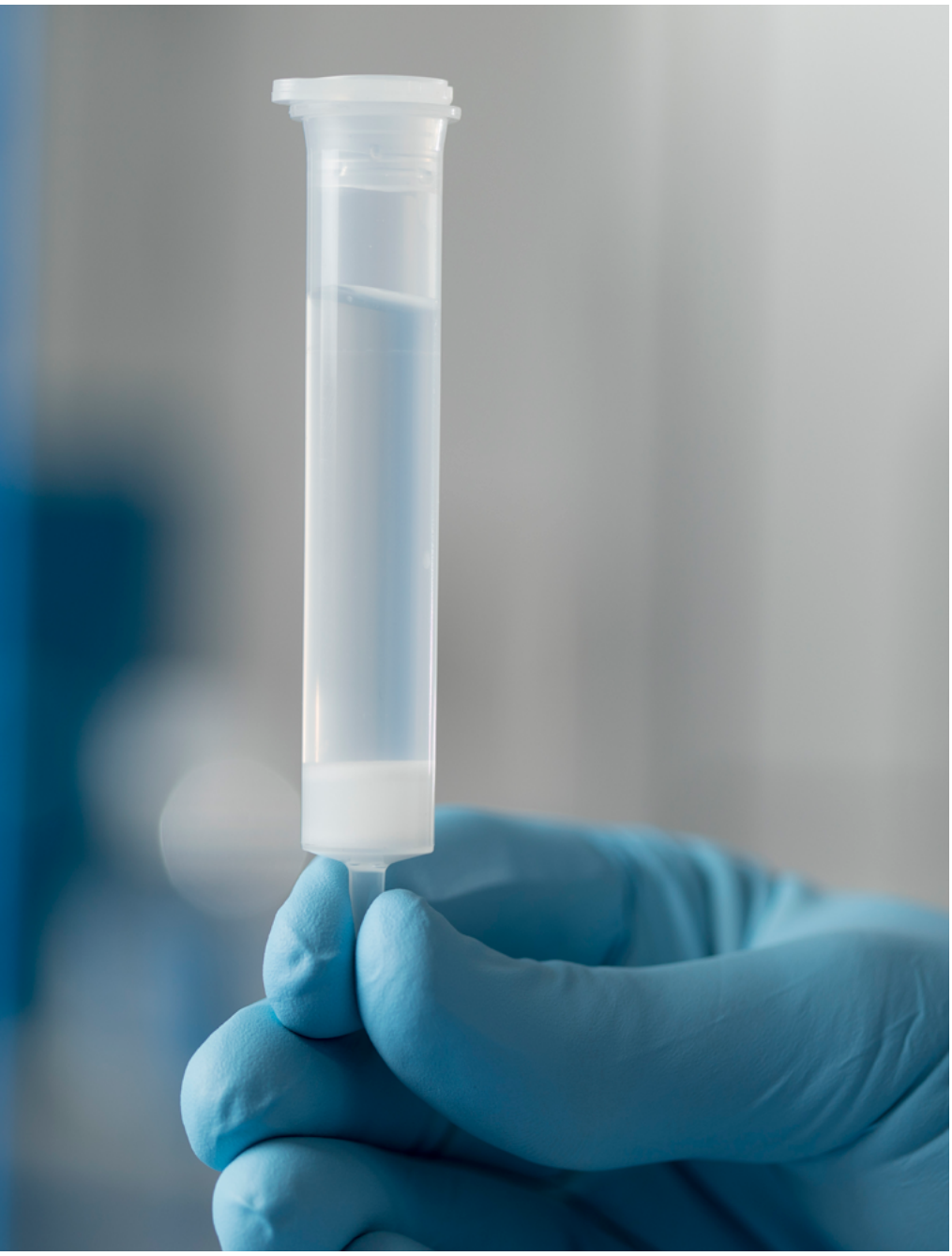
COMPREHENSIVE COMPARISON OF THE STREP-TAG® TECHNOLOGY WITH THE HIS-TAG SYSTEM



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THERE ARE VARIOUS WAYS OF SEPARATING A CERTAIN PROTEIN FROM OTHER CELLULAR COMPONENTS.

Affinity chromatography is one of the most efficient techniques. For this purpose, the desired protein is fused to a special peptide or protein (affinity tag), which in turn has a preference for a specific ligand. Such ligands can be other proteins, smaller molecules or metals that are immobilized on a chromatography matrix. While the affinity-tagged protein remains on the chromatography matrix via this affinity tag:ligand interaction, other cell components can be removed by washing. For the elution of the desired protein, the interaction between affinity tag and ligand is resolved by changing the buffer conditions, for example pH or substance concentration, or by adding a specific competitor that competes with the affinity tag for the ligand.

The principle of affinity chromatography is used for both the His-tag system and the Strep-tag® technology. The basis of the His-tag system is a small tag made up of six or more histidine residues in series. These residues can bind to transition metal ions – usually nickel or cobalt – which function as ligands. The Strep-tag® technology is based on the natural interaction between streptavidin and biotin. As affinity tag peptide serves either the Strep-tag®II (WSHPQFEK) or the Twin-Strep-tag®. The latter contains the Strep-tag® motif twice separated by a spacer. Both Strep-tags can bind to the biotin binding pocket of the engineered streptavidins, Strep-Tactin® and Strep-Tactin®XT. Elution in the Strep-tag® technology is triggered by the specific competitor biotin or by its analogue desthiobiotin. In contrast, elution in the His-tag system can be accomplished in three unspecific ways: 1) by lowering the pH (4.5–6), 2) by adding chelating agents (EDTA), or 3) with an imidazole gradient (20–250 mM). The use of chelating agents also means that the transition metal ions are detached from the matrix and have to be recharged after purification.

The well-known and repeatedly listed **attributes of the His-tag system** are:

- possibility to work under strongly denaturing conditions
- high yield
- associated low price.

These arguments seem extremely tempting – but only at first glance, because a closer look reveals some significant weaknesses compared to the **Strep-tag®** technology!

7 COMPATIBLE REAGENTS FOR HIS-TAG- AND STREP-TAG®-BASED PROTEIN PURIFICATION

It is necessary to work under denaturing conditions if membrane proteins or proteins present in inclusion bodies should be isolated. Both the Strep-tag® technology and the His-tag system allow the application of urea and guanidine (table 1). Furthermore, detergents such as Triton X-100, Tween 20 and Nonidet P40 can be added in order to increase the solubility of poorly soluble proteins. However, not all proteins are present in inclusion bodies after overexpression, and membrane proteins represent merely 20–30% of cellular proteins. Thus, it is not a requirement to choose the His-tag system from the outset only for the sake of denaturing conditions. Under these circumstances, it is much more important to recognize that the His-tag system is not compatible with many conventional buffers, salts, ligands, metal ions, and reducing or chelating agents. The addition of β-mercaptoethanol or calcium chloride is only possible to a small extent. Tris, HEPES or MOPS buffers are not recommended, while adding ammonium, DDT or EDTA should be completely avoided. However, all of the previously mentioned substances can be used in conjunction with the Strep-tag® technology without hesitation. Thereby, the Strep-tag® technology offers an incomparable protection, in which the target protein is stabilized by ligands or metal ions, the degradation by proteases is inhibited, and damage by oxidation is prevented.

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TABLE 1: Compatible reagents and representative values for His- or Strep-tag® based protein purification.

Reagents		His-tag system	Strep-tag® technology
Reducing agents	DTT	Not recommended	50 mM
	β-mercaptoethanol	Up to 20 mM	50 mM
	TCEP	Not recommended	10 mM
Denaturing agents	Urea	8 M	Up to 6 M
	Guanidine	6 M	Up to 1 M
Detergents	Triton X-100	2%	2%
	Tween 20	2%	2%
	Nonidet P40	2%	2%
Chelating agents	EDTA	Not recommended	50 mM
	EGTA	Not recommended	5 mM
Metal ion/ligand	$CaCl_2$	5 mM, maximum	Up to 1 M
Buffer components	Ammonium	Not recommended	2 M
	NaCl	Up to 2 M, at least 300 mM should be used	5 M
	Tris	Not recommended	Possible
	HEPES	Not recommended	Possible
	MOPS	Not recommended	Possible

This means that a large number of proteins can be addressed, such as metalloproteins, which should ultimately represent up to 50% of cellular proteins and which include enzymes, transcription factors and proteins for transport or storage. Furthermore, the His-tag system is also not suitable for proteins which prefer a low pH, since low pH values from 4.5–6 lead to elution instead of immobilization. As opposed to this, the Strep-tag® technology allows pH values from 4–10 and is therefore also convenient for pH-sensitive proteins.

The Strep-tag® technology allows the use of various substances, like metal ions, reducing and chelating agents.

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THE EXPRESSION HOST ISSUE – STREP-TAG® TECHNOLOGY IS THE ANSWER

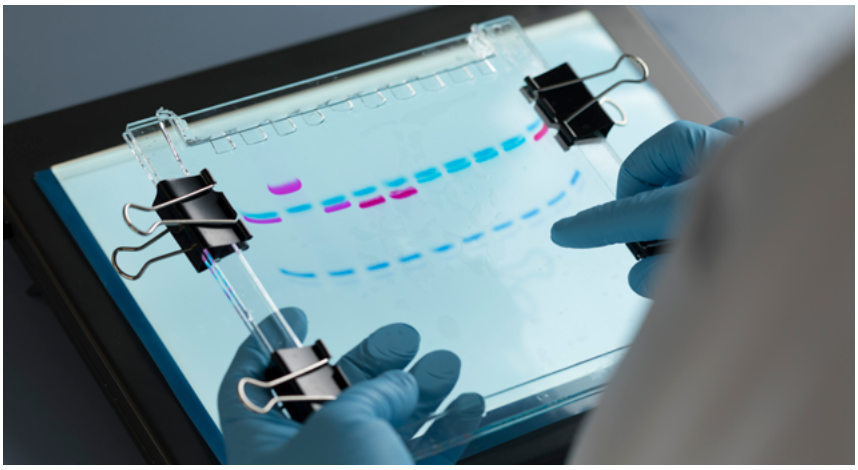
„Various experiments with biotin-containing media for mammalian expression have shown that the presence of the component does not affect the interaction between Twin-Strep-tag® and Strep-Tactin®XT".

Besides the limitations of the His-tag system towards various reagents, which can occasionally be crucial for the functionality of a target protein, additional problems arise when choosing the appropriate expression host. While barely sufficient results can be achieved with *E. coli* as expression host, it looks significantly worse in connection with yeasts, mammalian or insect cells – especially when secreted proteins should be purified. Yeast and insect cell media usually have an acidic pH, which interferes with the binding of His-tagged proteins to the immobilized metal chelate affinity chromatography (IMAC) resin. Moreover, media for yeast or mammalian cell cultivation often contain amino acids, such as histidine, glutamine or arginine, which compete with the His-tag for binding sites. The Strep-tag® technology is as well in this respect ahead of the competition. Neither an acidic pH nor free amino acids influences the binding of Strep-tagged proteins. Better still: with Strep-Tactin®XT as ligand, buffers and media with a pH value from 4–10 can be applied. Impairment of the Strep-tag® binding by biotin present in these media is no cause for concern.

Various experiments with biotin-containing media for mammalian expression have shown, that the presence of the component does not affect the interaction between Twin-Strep-tag® and Strep-Tactin®XT*. Those who still consider expression

* Source: Transient expression in mammalian cells (2019), IBA Lifesciences

in yeast, insect or mammalian cells with the His-tag system should be aware of the additional costs for dialysis, filtration, and size-exclusion or ion-exchange chromatography. In this way the initially high and inexpensive yield achieved with the aid of the His-tag system becomes a thing of the past.



03

THE DECISION: QUALITY OR QUANTITY?

Successful purification is the basis for the further characterization and analysis of the desired protein. But what determines a successful purification? If the protein is available in a sufficient quantity? Far from it! Quality in terms of purity and bioactivity is much more important than quantity. The His-tag system is said to have a high protein yield (5–40 mg/ml resin), but ideally generates a purity of 80%. If the function of contaminating proteins should not be measurable in the subsequent analyses, further cleaning steps are necessary. In this regard, the Strep-tag® technology has fully satisfied the requirements of one-step purification: a purity of more than 95% can be achieved with a simultaneous yield of about 16 mg/ml resin, which means that no further purification steps are necessary for the following applications.

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STREP-TAG® TECHNOLOGY – USE THE RIGHT AFFINITY!

In case you are still undecided on which system to choose, the following advice should be given. The His-tag system disposes only an affinity in the nM–µM range. This affinity leads to rapid dissociation and poor immobilization. In addition, His-tag antibodies have only a low specificity and can also detect unspecific proteins with His residues arranged in tandem. A large number of analytical applications for which a high affinity and/or highly specific antibodies are necessary – such as SPR (Surface Plasmon Resonance) or BLI (Bio-Layer Interferometry) – can only be addressed inadequately. The Strep-tag® technology by contrast offers an affinity in the µM–pM range. Depending on the application, the appropriate affinity can be selected. Further on, a large number of products are already available for the Strep-tag® technology, which allows a direct transition from protein purification to analytical application. To name just a few: antibodies and Strep-Tactin®XT conjugated with fluorescent dyes, Strep-Tactin®XT coated microplates or the Twin-Strep-tag® Capture Kit for SPR.

If you are already planning your experiments with the **Strep-tag®** technology, but still have some questions, just drop an email to **strep-tag@iba-lifesciences.com** We are here to help.

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SUMMARY

Reagents	His-tag system	Strep-tag® technology
Affinity tag	6 or more consecutive histidine residues	Strep-tag®II (8 AA peptide) or Twin-Strep-tag® (28 AA peptide)
Position	N-terminal	N-terminal
	Internal	Internal
	C-terminal	C-terminal
Ligand	Transition metal ions (nickel or cobalt)	Engineered streptavidins: Strep-Tactin® and Strep-Tactin®XT
Eluent	Imidazole gradient (0–250 mM)	Desthiobiotin
	Decrease in pH (4.5–6)	Biotin
	Chelation agents (EDTA)	
Re-use of resin	Yes	Yes
Good yields with	*E. coli*	*E. coli*
		Yeast
		Insect cells
		Mammalian cells

Reagents	His-tag system	Strep-tag® technology
Poor purification with	Yeast	
	Insect cells	
	Mammalian cells	
Suitable for	Membrane proteins	All protein classes, including membrane proteins, metalloproteins, pH sensible proteins, oxidation and proteolytic damage sensitive proteins
Not suitable for	Metalloproteins	-
	Proteins susceptible to oxidation or proteolytic damage	
	pH-sensitive proteins	
Purity	~80%	>95%
Yield	5–40 mg/ml resin	Up to 16 mg/ml resin
Native conditions	Yes	Yes
Denaturing conditions	Yes	Up to 6 M urea
Detergents	Yes	Yes
Chelating agents	Not recommended	Possible
Reducing agents	Not recommended	Possible
Salts	With restrictions	Possible
Affinity	µM–nM	µM–pM
Analytic applications	Not recommended for high affinity applications, like BLI or SPR	No limitations
Drawback	Interacts non-specifically with complex-forming amino acids	Interacts with biotinylated proteins, but they can be masked by addition of avidin