Strep-tag purification and Biotin contaminations

- How to block unspecific Biotin-

Version:1.0

Biotin content in cell culture media

Free Biotin binds to *Strep*-Tactin and thereby inactivates *Strep*-Tactin resins (biotin capacity ≅ 350 nmol /ml sedimented resin). It has to be removed or masked prior to affinity chromatography. This is mostly relevant when cell culture supernatant containing secreted recombinant protein is directly subjected to Strep-Tactin affinity chromatography because some media for insect cells or mammalian cells might contain significant amounts of biotin (see Fig.1).
The cell internal content of biotinylated proteins and free biotin is rather low and not a threat for significant inactivation of the Strep-Tactin resin in protein purification (see Fig.2). 1 µg biotin corresponds to 4 nmol.

Avidin for blocking Biotin

The simplest way to get rid of the biotin for purification of secreted eukaryotic/baculo proteins is irreversible masking by the addition of Avidin. Avidin will specifically bind to Biotin and not harm the Strep-tag/Strep-Tactin interaction. 1 U avidin blocks 1 µg biotin.

IBA biotin blocking products

IBA provides different products for biotin blocking, depending on what application it is used for.

For purification from cell culture supernatants with cell culture media containing Biotin we recommend our ready-to use **BioLock Biotin Blocking Solution** (Cat. No. 2-0205-050) with an activity of >70U/ml.
The Biotin content of several standard cell culture media and the required amount of blocking solution can be seen in Fig. 2.

For protein interaction experiments even the cell internal Biotin content (especially the biotinylated proteins) is of importance because they lead to unspecific (false positive) binding of these biotinylated proteins to Strep-Tactin. This can be avoided by adding our **high grade lyophilized Avidin** powder (Cat. No. 2-0204-015) with an activity of 11U/mg.
Add 1U avidin per µg contaminating biotin. The cell internal biotin contents can be seen in Fig.1.

Fig. 1: Cell internal biotin content of some organisms*:

Organism	**Biotin [µg]**
E. coli	1.75 **[l/OD]**
HEK-293 cells	0.5 [per 1x10^8 cells]
CHO cells	-

* is < 1% of the biotin capacity/ml column bed volume

Serum added may also contain biotin

However, serum we have tested (FCS, PAA) did not contain measurable amounts of biotin (<0.025 µg/ml; <0.1 µM).
Ingredients of proprietary formulations for serum free growth are usually not disclosed but information on biotin content can be obtained from the respective manufacturer upon request (these media are likely to contain biotin as well).

Fig.2: Overview on Biotin content of cell culture media

Medium	Manufacturer	Biotin content [µg/L]	Required amount of BioLock solution per Liter medium *** [ml]
mammalia			
BME (Eagle)[1]	Multiple Suppliers	1000	15.7
CMRL 1066[2]	Multiple Suppliers	10	0.2
FreeStyle™ CHO Expression Medium *	Gibco® (Cat. No.12651-014)	1759	27.7
DMEM/F-12	Multiple Suppliers	35	0.6
Hams F10[3]	Multiple Suppliers	24	0.4
Hams F12[4]	Multiple Suppliers	7	0.1
ExCell ® 302 CHO**	SAFC (Cat. No.24324C)	110	1.7
Expi293™	Gibco® (Cat. No. A1435101)	1151	18.1
FreeStyle™ 293*	Gibco® (Cat. No.12338-018)	100	1.6
FreeStyle ™ F 17*/**	Gibco® (Cat. No. A13835-01)	684 / 484	10.7 / 7.6
Fischer's Medium[5]	Multiple Suppliers	10	0.2
Iscove's (IMDM)	Multiple Suppliers	13	0.2
McCoys 5A	Multiple Suppliers	200	3.1
MCDB 131	Multiple Suppliers	7.3	0.1
Medium 199[6]	Multiple Suppliers	10	0.2
MEM α	Multiple Suppliers	100	1.6
NCTC 109/135	Multiple Suppliers	25	0.4
RPMI 1640[7]	Multiple Suppliers	200	3.1
Waymouth's MB 752/1	Multiple Suppliers	20	0.3
Williams' Medium E[8]	Multiple Suppliers	500	7.9
insect cells			
Express Five® SFM**	Gibco® (Cat. No.10486-025)	147	2.4
EX-CELL® 405**	Sigma (Cat. No.14405C)	73	1.2
EX-CELL® 420**	Sigma (Cat. No.14420C)	186	3.0
Insect-XPRESS™**	Lonza (Cat. No.12-730F)	147	2.4
Sf-900™ II SFM**	Gibco® (Cat. No.10902-096)	149	2.4
Sf-900™ III SFM**	Gibco®(Cat. No.12658-027)	150	2.4
SF3-Baculo Express**	Promocell (Cat. No. C-783-10)	110	1.7
HyClone® HyQ® SFX-Insect™**	Thermo Scientific HyClone (Cat. No.SH3027801)	180	3.0

[1] Eagle H. (1965), Proc. Soc. Exp. Med. 89, 362; [2] Parker, R.C., et al. (1957) Special Publications, N.Y. Academy of Sciences, 5, 303; [3] Ham, R.G. (1963), Exp. Cell Res., 29, 515; [4] Ham, R.G. (1965), Proc. Nat. Acad, Sci., 53, 288; [5] Fischer, G.A. and Sartorelli, A.S. (1964), Methods in Med. Res. 10; [6] Morgan, Morton and Parker (1950) Proc. Soc. Exp. Biol. Med., 73, 1;
[7] Moore, G.E., Gerner, R.E. and Franklin, H.A. (1967) A.M.A. 199, 519; [8] Williams, G.M. and Gunn, J.M. (1974) Exp. Cell. Res., 89, 39
* Manufacturer data; ** IBA internal measurement; ***the calculated volume includes a 10% excess

Biotin free media

Medium	Manufacturer	Biotin content [µg/L]	Required amount of Biotin blocking solution per Liter medium [ml]
mammalia			
DMEM[9]	Multiple Suppliers	-	-
Leibovitz's L-15[10]	Multiple Suppliers	-	-
ProCHO™ 5**	Lonza (Cat. No.12-766Q)	-	-
ExCell 293 HEK	Sigma (Cat. No.14571C)	-	-
insect cells			
Graces Insect Medium**	Gibco (Cat. No.11605-045)	-	-
Schneider's Medium[11]	Multiple Suppliers	-	-

[9] Schneider, I. (1964), Exp. Zool. 156, 1 , 91; [10] Leibovitz, A. (1963) Am. J. Hyg. 78, 173; [11] Dulbecco, R. Freeman, G. (1959) Virology 8, 396. Smith, J.D., Vogt, M. and Dulbecco, R. (1960) Virology 12, 185

* Manufacturer data

** IBA internal measurement

How to use BioLock?

After cell culture, remove the cells by centrifugation (200 g)
Add 0.1 volumes 10x buffer W (e.g. for 1000ml culture 100ml buffer W (10x)) and the necessary amount of BioLock solution (see table above).
After 15 min incubation, clear supernatant by further centrifugation step (10.000 g).
Apply the cleared supernatant to the gravity flow column.

Further methods to remove biotin:

- to precipitate the recombinant protein in a first step by ammonium sulfate precipitation, then to remove the biotin containing supernatant and finally to dissolve the precipitated protein prior to Strep-tag chromatography with buffer W (100 mM Tris-Cl pH 8.0; 150 mM NaCl; 1 mM EDTA (EDTA can be omitted in case of metalloproteins)).

- to make a crude ion exchange step with elution at slightly alkaline pH (>7.5) for direct application on a Strep-Tactin column.

- to concentrate the protein by cross flow ultrafiltration. Please use buffer W for exchange so that the protein concentrate can be applied directly to a Strep-Tactin column.

Although more labour-intensive than adding avidin, these procedures have the advantage that the recombinant protein will be concentrated which contributes to stability of the recombinant protein and which enables higher efficiency of Strep-tag affinity chromatography.

pH > 7.5 is necessary for efficient Strep-tag chromatography and has in every case to be respected. Precipitates may form during masking with avidin or during concentration steps and have to be removed prior to Strep-tag affinity chromatography.