Solutions For Life Sciences

Data Sheet

BioLock-Biotin blocking solution

Cat. No.: 2-0205-050, 2-0205-250
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Description	Culture media often contain significant amounts of biotin. This is especially the case for mammalianor insect cell culture media. Thus, if proteins from biotin containing extracts or media are intended to be purified via Strep-Tactin® chromatography, biotin must be masked by the addition of avidin prior to the application onto the column. **Add at least 1U of BioLock solution per µg of biotin**. However, the biotin concentration in most cell culture supernatants (including Expi media) is not critical for the binding capacity of Strep-Tactin®XT and supernatant can be loaded directly on the resin. Information on the biotin content of media and hosts can be obtained from the attached flyer.
Activity	>70 U/ml (determined by HABA dye binding method) Unit definition: 1 U avidin blocks 1 µg biotin
MW	~17000 Da (monomer)
Stability	12 months after shipping
Storage	Reagent is stable at room temperature, but does not contain any preservatives. Therefore, please aliquot and store at -20°C or aliquot under sterile conditions and store at room temperature. Protect from light.
Shipping	room temperature
Hazards	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

Application	- After cell culture, remove the cells by centrifugation (300 x g, 10 min) - Add 0.1 volumes 10x Buffer W (Cat. No. 2-1003-100) (e.g. for 1000ml culture add 100ml Buffer W) to adjust the pH to 8.0, then add the necessary amount of BioLock solution (see information below for standard biotin concentrations of mammalian and insect cell media). **Note:** pH of the cell culture supernatant should be >7.0 (recommended: 8.0) before it is applied to the Strep-Tactin® column to allow for efficient Strep-tag® or Twin-Strep-tag® binding. - After 20 min incubation, clear supernatant by a further centrifugation step (10.000 x g, 20 min). - Subject the cleared supernatant to Strep-Tactin® affinity chromatography.
Notes	In addition to avidin, this solution contains substantial amounts of lysozyme and ovotransferrin and further traces of other egg white proteins which do not interfere with Strep-tag® purification. **Any occurring turbidities are not a quality defect and should be removed via centrifugation prior to use.**

For research use only

Strep-tag® purification and Biotin contaminations

- How to block unspecific Biotin-

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,-0250) with an activity of >70 U/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, -050) with an activity of 11 U/mg.
Add 1 U 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 content
E. coli	1.75 [µg/L*OD]
HEK-293 cells	0.5 [µg 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	3.5	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

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 labor-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.

Note: In case Biotin has already bound, BioLock enables the affordable removal of biotin from surfaces/matrices with reversible biotin binding affinity (regeneration) through an appropriate incubation and subsequent washing step. Equilibrium will be shifted to the avidin-biotin complex driven by its extraordinary affinity in the femtomolar range. A sufficient off-rate of the biotin binding interaction on the surface/matrix in correlation with the incubation time of BioLock is nevertheless a prerequisite for efficient regeneration.