Protein Production & Assays

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Protocol

Blocking of Biotin in Cell Lysates and Culture Media

1. GENERAL INFORMATION

Cell culture supernatants often contain high amounts of free biotin. These are unproblematic when working with Strep-Tactin®XT resins since biotin does neither bind irreversibly to this ligand nor reduces the binding capacity. However, this is the case for the application of resins coupled to Strep-Tactin®. Free biotin can bind to the engineered biotin binding pocket of Strep-Tactin® and prevents the binding of Strep-tag®II or Twin-Strep-tag® proteins. Therefore, biotin must be removed or masked prior to protein purification. The cell internal content of free biotin is rather low and not a threat for significant inactivation of the Strep-Tactin® resin in protein purification.
Besides free biotin, cell lysates also contain small amounts of biotinylated proteins. Normally, these proteins do not influence the purification results with Strep-Tactin® or Strep-Tactin®XT due to their low abundance. However, when it comes to analytic applications with high sensitivity, impurities should be prevented.
The simplest way to get rid of biotin or biotinylated proteins prior to protein purification is irreversible masking by the addition of avidin. Avidin is a tetrameric biotin binding protein (K_D for biotin > 10^{-14}) extracted from egg white, which does not bind to Strep-tag®II or Twin-Strep-tag®. 1 U avidin blocks 1 µg biotin.

2. Biotin Blocking Products

Depending on the application, IBA provides different products for biotin blocking. For protein purification from cell culture supernatants, we recommend the application of BioLock, a ready-to-use biotin blocking solution (Cat. No. 2-0205-050 and 2-0205-250) with an activity of >70 U/ml.
For protein interaction experiments, especially biotinylated proteins are of importance since they can bind to Strep-Tactin® as well as Strep-Tactin®XT and lead to false positive interactions. This can be avoided by adding our high grade lyophilized avidin powder (Cat. No. 2-0204-015) with an activity of 11 U/mg.

3. Application of BioLock

3.1 After cell culture, remove the cells by centrifugation (300 x g, 10 min)

3.2 Add 0.1 volumes 10x Buffer W (e.g., for 1000 ml culture 100 ml 10x Buffer W) and the necessary amount of BioLock solution. Known biotin concentrations of organisms and cell culture media are listed under section “4. Biotin content of organisms and cell culture media”.

3.3 After 20 min incubation, clear supernatant by centrifugation (> 3000 x g, 20 min).

3.4 Apply the cleared supernatant to the gravity flow column and proceed with conventional protein purification. Please note that the pH of the sample should be 7-8 before it is applied to the Strep-Tactin® resin to allow efficient Strep-tag®II or Twin-Strep-tag® binding.

4. Biotin Content of Organisms and Cell Culture Media

The cell internal biotin content of some organisms is mentioned below. However, it is mostly lower than 1% of the biotin binding capacity per ml column bed volume. The biotin capacity of Strep-Tactin® and Strep-Tactin® high capacity resins is approximately 350 and 900 nmol/ml resin, respectively.

Organism	Biotin content
E. coli	1.75 µg biotin per liter culture at OD_{600}
HEK-293 cells	0.5 µg biotin per 1 x 10^8 cells
CHO cells	-

Applied Serum 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).
Mammalian and insect cell culture media contain different amounts of biotin. An overview of known biotin concentrations is listed below.

Medium	Manufacturer	Biotin content (µg/l)	Required amount of BioLock solution per Liter medium (ml)***
Mammalia cell culture media			
BME (Eagle)[1]	Multiple Suppliers	1000	15.7
CMRL 1066[2]	Multiple Suppliers	10	0.2
DMEM[9]	Multiple Suppliers	-	-
Hams F10[3]	Multiple Suppliers	24	0.4
Hams F12[4]	Multiple Suppliers	7	0.1
ExCell® 293 HEK	Sigma (Cat. No.14571C)	-	-
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™ F17*/**	Gibco® (Cat. No. A13835-01)	684/484	10.7/7.6
FreeStyle™ CHO Expression Medium*	Gibco® (Cat. No. 12651-014)	1759	27.7
Fischer's Medium[5]	Multiple Suppliers	10	0.2
Iscove's (IMDM)	Multiple Suppliers	13	0.2
Leibovitz's L-15[10]	Multiple Suppliers	-	-
MCDB 131	Multiple Suppliers	7.3	0.1
Medium 199[6]	Multiple Suppliers	10	0.2
MEM α	Multiple Suppliers	100	1.6
MEXi-CM	IBA Lifesciences GmbH	120	1.9
MEXi-TM	IBA Lifesciences GmbH	-	-
NCTC 109/135	Multiple Suppliers	25	0.4
ProCHO™ 5**	Lonza (Cat. No. 12-766Q)	-	-
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 cell culture media			
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
Graces Insect Medium**	Gibco® (Cat. No. 11605-045)	-	-
HyClone® HyQ® SFX-Insect™**	HyClone (Cat. No. SH3027801)	180	3.0
Insect-XPRESS™**	Lonza (Cat. No. 12-730F)	147	2.4
Schneider's Medium[11]	Multiple Suppliers	-	-
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

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 Lifesciences internal measurement
*** the calculated volume includes a 10% excess

5. Further Methods to Remove Biotin

Ammonium Sulfate Precipitation	Precipitate the recombinant protein in a first step by ammonium sulfate. Remove the biotin containing supernatant and finally dissolve the precipitated protein prior with 1x Buffer W (100 mM Tris-Cl pH 8.0; 150 mM NaCl; 1 mM EDTA (EDTA can be omitted in case of metalloproteins)).
Cross Flow Ultrafiltration	Perform conventional cross flow ultrafiltration and use 1x Buffer W for exchange so that the protein concentrate can be applied directly to a Strep-Tactin column.
Dialysis	Dialyze with an appropriate dialysis product according to the manufacturer information 3 times 1:100.
Ion Exchange	Perform conventional ion exchange and elute at slightly alkaline pH (>7.5) for direct application on Strep-Tactin®.
Size exclusion chromatography	Use a size exclusion chromatography matrix with an appropriate fraction range, e.g., 1000-5000 Da, according to the manufacturer information.

Please note that after biotin removal the sample should have a pH > 7.5 for efficient Strep-Tactin® based protein purification afterwards. Precipitates that have been formed during the process have to be removed prior to protein purification.

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If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!

IBA Lifesciences GmbH · Rudolf-Wissel-Str. 28 · 37079 Goettingen · Germany · Phone + 49 551 50672-0 · info@iba-lifesciences.com