

## Biotin content in cell culture media

Free Biotin inactivates *Strep*-Tactin resins (biotin capacity  $\cong$  350 nmol/ml sedimented resin) and 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 contain significant amounts of biotin. 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. 1 mg biotin corresponds to 4  $\mu$ mol.

The simplest way to get rid of the biotin problem for purification of secreted eukaryotic proteins is irreversible masking by the addition of avidin (IBA cat no 2-0204-050). Use **120 mg avidin per mg contaminating biotin**. This procedure is recommendable at high expression levels

Other possibilities are:

- 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.

For mammalian cell culture only DMEM and Leibovitz's L-15 media are free of biotin. The same is true for Schneider's medium and for Graces from Gibco/Invitrogen for insect cell culture. Serum added may also contain biotin. However, serum we have tested (FCS, PAA) did not contain measurable amounts of biotin (<0.025  $\mu$ g/ml; <0.1  $\mu$ M). 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).

Biotin contents of standard mammalian cell culture media:

Medium	D-Biotin [mg/l]	Citation
BME (Eagle)	1	Eagle H. (1965), Proc. Soc. Exp. Med. 89, 362
CMRL 1066	0.01	Parker, R.C., et al. (1957) Special Publications, N.Y. Academy of Sciences, 5, 303

DMEM	-	Dulbecco, R. Freeman, G. (1959) Virology 8, 396. Smith, J.D., Vogt, M. and Dulbecco, R. (1960) Virology 12, 185
DMEM/F-12	0.035	
Hams F10	0.024	Ham, R.G. (1963), Exp. Cell Res., 29, 515
Hams F12	0.007	Ham, R.G. (1965), Proc. Nat. Acad, Sci., 53, 288
Fischer's	0.01	Fischer, G.A. and Sartorelli, A.S. (1964), Methods in Med. Res. 10.
Iscove's (IMDM)	0.013	
Leibovitz's L-15	-	Leibovitz, A. (1963) Am. J. Hyg. 78, 173
McCoy's 5A	0.2	
MCDB 131	0.0073	
Medium 199	0.01	Morgan, Morton and Parker (1950) Proc. Soc. Exp. Biol. Med., 73, 1
$\alpha$ MEM	0.1	
NTCC 109/135	0.025	
RPMI 1640	0.2	Moore, G.E., Gerner, R.E. and Franklin, H.A. (1967) A.M.A. 199, 519
Waymouth's MB 752/1	0.02	
Williams' E	0.5	Williams, G.M. and Gunn, J.M. (1974) Exp. Cell. Res., 89, 39

Biotin contents of standard insect cell culture media:

Medium	D-Biotin [mg/l]	Citation
Grace's	0.01	Grace, T.C. (1962) Nature 195, 788
Schneider's	-	Schneider, I. (1964), Exp. Zool. 156, 1, 91
SF900II from Invitrogen	0.1	determined by IBA
SF3 from PromoCell	0.1	determined by IBA
ExCell 401 from JRH Biosciences	0.1	determined by IBA
Express Five from Gibco/Invitrogen	0.15	determined by IBA
Graces from Gibco/Invitrogen	-	determined by IBA
Insect Xpress from Cambrex	0.1	determined by IBA