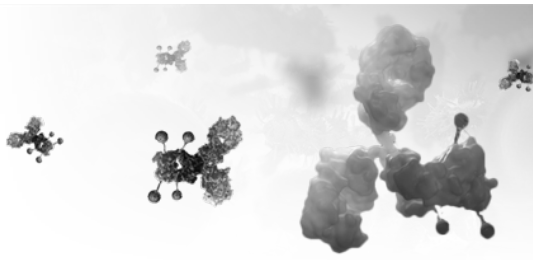

Vitamin B12 Immunoaffinity Column (CDM040412)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Size	10 Columns
Detection Limit	0.1 to 5µg/g vitamin B12
Assay Procedure	Sample Preparation: Vitamin B12 samples are to be extracted and analysed with the method of Li et al. [H.-B. Li, F. Cheng, Y. Jiang J. Chromatogr. A 2000; 891:243- 247], e.g. vitamin tablets, liquid vitamin preparations, cell culture extracts. Example: 25g vitamin containing tablets are dissolved in 100ml PBS. The resulting extract may be filtered through a 0.45µm membrane filter. Enrichment Step IAC: 4ml extract (containing the quantity of Biotin from a 1g sample if above-mentioned sample preparation is followed) is diluted with a total volume of 20ml PBS and then applied in a reservoir on top of the BioTeZ-Immunoaffinity Column. The optimal flow rate through the gel is between 1 to 3ml/min. According to application and contents expected the applied extract volumes could vary. E.g. extracts may be diluted 1+1 with PBS or 1+4 as mentioned above. In case of very low contents even extract volumes of 200ml may be applied without significant loss of analyte as long as resulting pH is fairly neutral and alcohol or acetonitrile content lies under 15%. Wash: After the whole sample has passed through the gel, the latter is washed with 5ml of PBS. Remaining liquids in the gel are removed by applying either pressure from top of the column or under-inflation from the bottom. Elution: The sample reservoir on top of the BioTeZ- Immunoaffinity Column is removed, and an appropriate vial is placed below the affinity column. The bounded vitamin B12 is eluted by using a total volume of 3ml of HPLC grade methanol. The elution process is performed in two steps.

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	First, an amount of 1ml methanol is applied. Once this amount has passed through the column, there should be a waiting time of 30 seconds. After that, the second portion of 2ml of methanol is eluted through the column. The remaining methanolic solutions should be eluted by application of slight under- or overpressure. All methanolic fractions are unified to give the column elute. The column elute may be injected into the HPLC directly or, if concentrations are very low, concentrated by evaporation (e.g. using VLM evaporator), re-dissolved in HPLC solvent and finally injected into the system. For the latter case, please see the sample calculation in which the sample concentrate is re-dissolved in 0.4ml HPLC solvent.
Recovery	Recovery rates are >85% when vitamin B12 in buffer mixtures is analysed in the range of 0.1 to 5µg per IAC.
Analytical Method	Machine: Shimadzu; Column: Trentec Reprosil- Pur RP C18 120 ODS3 5µm; 125x3,0mm with guard column; Mobile Phase A: acetonitrile /water (70:30 v/v) (use only for cleaning purposes at the beginning and at the end of analytical series); Mobile Phase B: 0.03M potassium phosphate, pH 7.0-methanol (80/20 v/v); Gradient: 0.01min B 100%; 30min B 100% (isocratic); Flow Rate: 0.5ml/min; Time of Analysis: 30min; Injector Volume: 100µl; Detection: λ ABS [nm]: 361nm.

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