

## Intended use:

B12Star™ immunoaffinity columns contain monoclonal antibodies against vitamin B12 (cyanocobalamin), which are covalently bound to gel-particles and are intended for the analysis of various commodities (e.g. vitamin tablets, liquid vitamin preparations, cell culture extracts).

## Recommended solvents and buffers:

- Extraction solution: PBS-buffer (10 mM)
  - 8 g NaCl, 1.2 g Na<sub>2</sub>HPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 0.2 g KCl (p.A.)
  - dissolve in 990 mL distilled or deionized water
  - adjust pH to 7.4 using NaOH (1 M) or HCl (1 M)
  - fill up to 1000 mL with distilled or deionized water
- Dilution: PBS-buffer (10 mM)
- Rinse solution: PBS-buffer (10 mM)
- Eluent: methanol (HPLC grade)

**All solvents and buffers should be at room temperature (15 – 25 °C).**

**Romer Labs recommends the use of Biopure™ isotope labeled internal standards.**

## Storage:

Always store at 2 – 8 °C (35 – 46 °F) when not in use.

Do not freeze. Do not use the IAC beyond the expiration date.

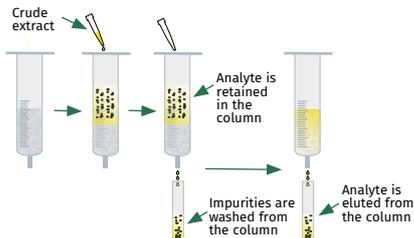
**IMPORTANT:** Download certificate of analysis by scanning the QR code on the external label or by visiting <https://www.romerlabs.com/en/customer-resources/>

## Special Notes for column use:

- StarLine™ IAC contain sodium azide.
- StarLine™ IAC are designed for single use only.

## Disclaimer/Warranty:

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## Procedure

### 1 Sample Preparation

- Vitamin B12 samples should be extracted with the method published by Li et al. [H.-B. Li, F. Cheng, Y. Jiang J. Chromatogr. A 2000; 891:243-247]. This method applies for vitamin tablets, liquid vitamin preparations and cell culture extracts.
- For example:
  - Weigh 25 g of vitamin containing tablets.
  - Add 100 mL PBS.
  - Filter extract through a 0.45 µm membrane filter.

### 2 Dilution

- 4 mL of extract (containing 1 g of sample if above-mentioned sample preparation is followed) are diluted with 20 mL PBS.
- For special applications, extracts can be diluted 1:1 with PBS.

### 3 Sample Application

- The IAC must be at room temperature (15 – 25 °C) for usage!
- It is not necessary to rinse the IAC before applying the extract!
- Apply the diluted extract to the IAC.
- For samples with a low concentration of vitamin B12, a volume of extract up to 200 mL can be applied to B12Star™ without a significant loss of analyte.
- Let all extract pass through the IAC with a flow rate of approx. 1 – 3 mL/min.

### 4 Rinse

- Rinse the IAC with 5 mL PBS at a flow rate of 1 – 3 mL/min.
- Remove liquid from the column by

### 5 Elution

- Place a suitable vial under the B12Star™ IAC.
  - To elute bound vitamin B12 apply 1 mL of methanol and wait until it has passed through the column!
  - Apply 2 mL of methanol and let it pass through the column (flow rate 1 – 3 mL/min).
  - Remove any remaining liquid from the IAC by applying pressure at the top or vacuum at the bottom.
  - In case of low level contamination the eluent can be dried down and re-dissolved in a small portion of mobile phase.
  - Inject
- applying pressure to the top or vacuum to the bottom. The column must not dry out completely!

You can find worldwide contact information and learn more about our complete line of products for mycotoxin testing on our website.