

Application Protocol: Determination of Pentraxin 3 (PTX3) in Stool **Extract with Human Pentraxin 3 (PTX3) ELISA, Cat.No. RD191477200R**

1. STOOL COLLECTION AND EXTRACTION

Collect 50 to 100 mg of stool for extraction procedure. Add BioVendor Extraction Buffer (Cat. No.: C005821) to a polypropylene tube with known weight of stool sample giving dilution factor 50x, e.g. if stool weight is 55 mg, add 2695 µl of Extraction Buffer [55 (weight) x 50 (dilution factor) – 55 (weight) = 2695 µl].

Homogenize the samples on a vortex at high speed for 30 minutes and centrifuge for 5 minutes at 3000xg. Use supernatant for analysis in ELISA.

Alternatively, it is possible to use Calprotectin Extraction Device (offered by BioVendor – Laboratori Medicina, Cat. No.: B-CAL-RD) for sample collection. The average weight of a stool sample drawn with the device is 87 mg; to achieve the extraction dilution factor of 50, add 4263 µl of the BioVendor Extraction Buffer Cat. No.: C005821 (instead of B-CAL-EX mentioned in the instructions). Homogenize the samples for 1 min on a vortex mixer until no large particles can be seen and centrifuge for 5 minutes at 3000xg. Use supernatant for analysis in ELISA.

Or, it is possible to use Stool preparation system (Immundiagnostik, Cat. No.: K 6998SAS) for sample collection. The average weight of a stool sample is 15 mg; to achieve the extraction dilution factor of 50, add 735 µl of the BioVendor Extraction Buffer Cat. No.: C005821. Homogenize the samples for 1 min on a vortex mixer until no large particles can be seen and centrifuge for 5 minutes at 3000xg. Use supernatant for analysis in ELISA.

2. ASSAY PROCEDURE

Recommended starting dilution for stool extract is 3-fold, e.g. 50 µl of stool extract (supernatant) + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of stool extract (supernatant) + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Follow the procedure described in Product Data Sheet.

Calculations:

The measured concentration of stool extract sample calculated from the standard curve must be multiplied by the respective extraction dilution factor and the respective ELISA dilution factor, e.g. 400 pg/ml (from the standard curve) x 3 (ELISA dilution factor) x 50 (extraction dilution factor) = 60 000 pg/ml = 60 ng/ml = 0,06 µg/g of stool sample.