Application Protocol: Determination of S100A12 in Stool Extract with Human S100A12 ELISA, Cat.No. RD191221200R

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 – Laboratorni 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 20-fold, e.g. 10 µl of stool extract (supernatant) + 190 µl of Dilution Buffer for singlets, or preferably 15 µl of stool extract (supernatant) + 285 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. 1 ng/ml (from the standard curve) x 20 (ELISA dilution factor) x 50 (extraction dilution factor) = 1 000 ng/ml = 1 µg/ml = 1 µg/g of stool sample.