

## **Application Protocol: Determination of Lipocalin-2 in Stool Extract with Human Lipocalin-2/NGAL ELISA, Cat.No. RD191102200R**

### **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  $[55 \text{ (weight)} \times 50 \text{ (dilution factor)} - 55 \text{ (weight)} = 2695 \text{ µl}]$ .

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 10-fold, e.g. 12 µl of stool extract (supernatant) + 108 µl of Dilution Buffer for singlets, or preferably 24 µl of stool extract (supernatant) + 216 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.  $1 \text{ ng/ml (from the standard curve)} \times 10 \text{ (ELISA dilution factor)} \times 50 \text{ (extraction dilution factor)} = 500 \text{ ng/ml} = 0,5 \text{ µg/ml} = 0,5 \text{ µg/g of stool sample}$ .