EDI™ Quantitative Fecal Calprotectin ELISA
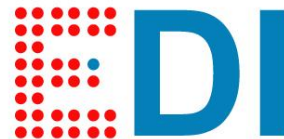
KT-849 & KT-843

CE

EDI
Epitope Diagnostics, Inc.

EDI™ Quantitative Calprotectin ELISA
Catalog No. KT-849

Intended Use:

- Quantitative determination of fecal calprotectin (neutrophil cytoplasmic protein S100A8/A9) concentration.

EDI™ Quantitative Calprotectin ELISA
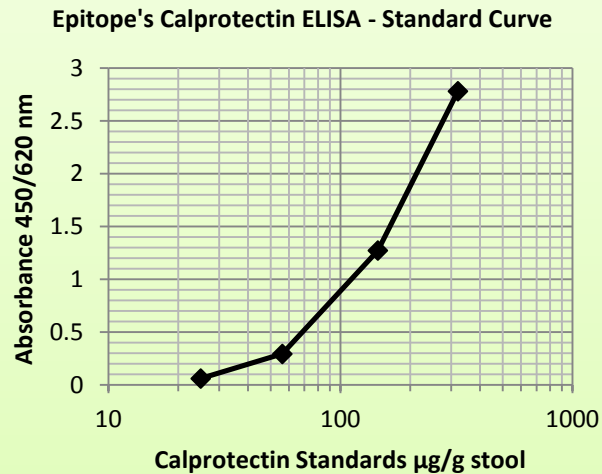### Catalog No. KT-849

Indication of Use:

- Diagnosis, monitoring of the severity of inflammatory bowel disease (IBD).
- Higher stool calprotectin levels are associated with an increased risk of relapse in patients with IBD.
- Low stool calprotectin levels correlate well with a low risk for intestinal allograft rejection.

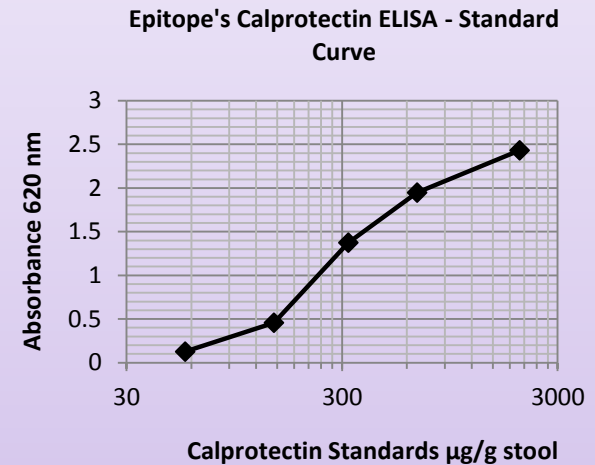# A Wide Measurement Range: 1 – 2000 µg/g stool

Reduces repeated measurement for patients with higher calprotectin

EXAMPLE STANDARD CURVE (low)

Epitope's Calprotectin ELISA - Standard Curve

EXAMPLE STANDARD CURVE (high)

Epitope's Calprotectin ELISA - Standard Curve
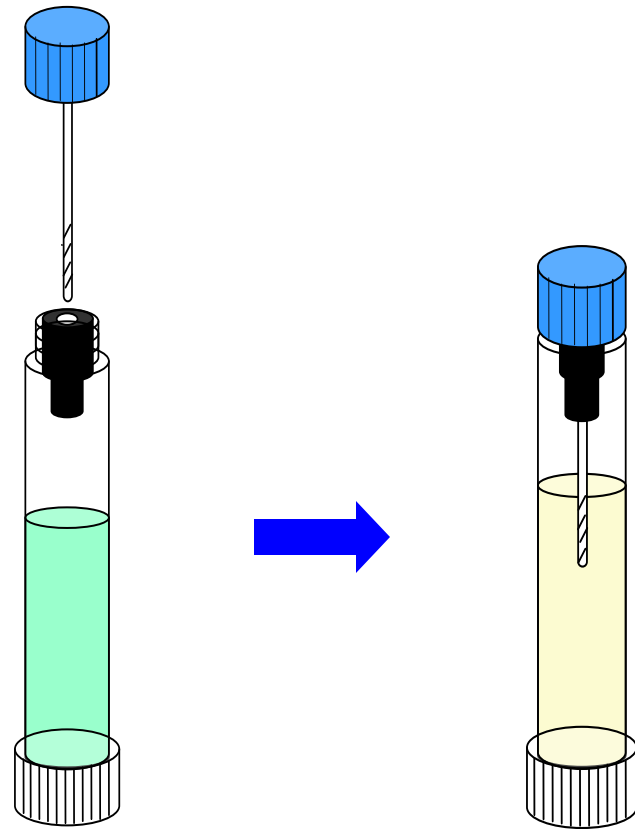
Convenient Fecal Sample Collection

- **No weighing of stool sample necessary**

- **No centrifugation**

- **Reduces the probability of lab contamination**

- **Reduces hazardous exposure to lab staff**

Simple Assay Procedures

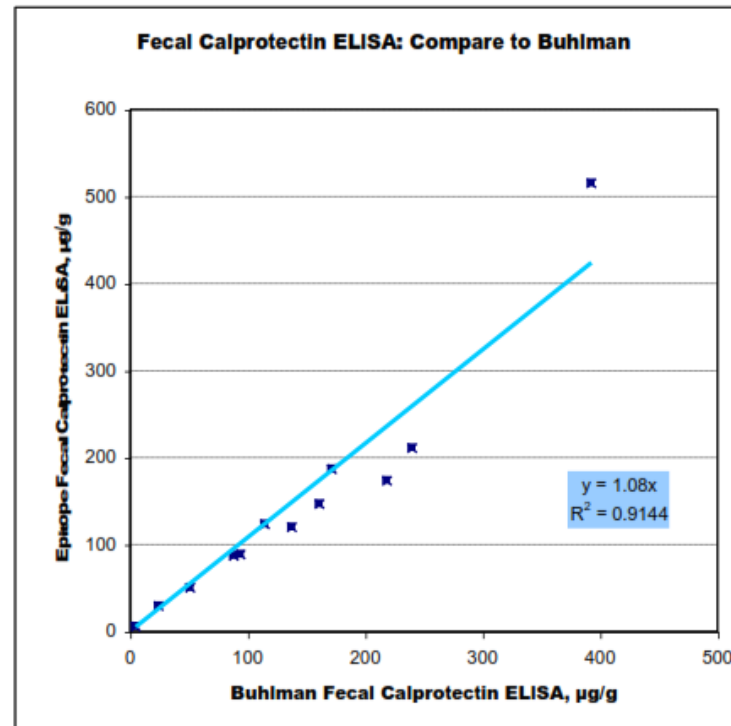- 60 min – 45 min – 12 min incubation
- Room temperature

- Ready on DS2, DSX or other automated open ELISA system.

Assay Comparison

Fecal Calprotectin ELISA Assay Comparison   11/2/2010

Sample	Buhlman (µg/g)	EDI (µg/g)
1	161.3	146.6
2	240	210.9
3	94	88.4
4	51.3	50.2
5	88.3	86.9
6	24.3	29.3
7	4.8	5.6
8	218.8	173.1
9	137.7	119.9
10	392.5	515.4
11	171.7	186.6
12	114.8	123.2
mean	141.625	144.7
R =	0.96	
R^2 =	0.93	

Fecal Calprotectin ELISA: Compare to Buhlman

$y = 1.08x$
$R^2 = 0.9144$

Epitope Fecal Calprotectin ELISA, µg/g

Buhlman Fecal Calprotectin ELISA, µg/g

Normal Cut Off

43.2 µg/g stool

Stool samples from normal healthy adults with ages of 24 – 58 were collected and measured with this ELISA. The recommended **normal cut-off** for fecal Calprotectin concentration by using this ELISA and sample collection system is **120 ng/mL or 43.2 µg/g directly read from assay standard curve**. We strongly recommend that each clinical laboratory establish its own normal cut-off level by measuring normal stool samples with this ELISA and sample collection system.

Calprotectin ng/mL X 0.36 = Calprotectin µg/g

Calprotectin µg/g X 2.78 = Calprotectin ng/mL

Sensitivity

- Analytical sensitivity (LLOD): 2.5 ng/mL, determined by the 95% confidence limit on 12 duplicate determination of zero standard is approximately.

- LLOQ: 5 ng/mL, determined by dilution of assay standards.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" for calprotectin level up to 40,000 ng/mL in extraction buffer.

Precision

The intra-assay precision was validated by measuring three sample extracts in a single assay with 12 replicate determinations.

Mean Calprotectin Value (µg/g)	CV (%)
5.74	2.9
26.59	3.5
54.70	2.5

The inter-assay precision was validated by measuring two samples in duplicate in 4 individual assays.

Mean Calprotectin Value (µg/g)	CV (%)
21.64	8.6
70.31	2.0

The precision of inter-sample collection was performed by collecting five specimens from one bowel movement. These grouped samples are measured in an assay according to the assay procedure. The results of Calprotectin concentration in the value of ng/mL indicate that there are very satisfactory agreements of the five samples collected from one bowel movement.

Donor	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	CV%
A	57.0	65.0	59.2	56.2	49.8	9.5
B	60.4	55.3	58.8	71.7	81.1	16.3
C	72.3	69.3	51.5	65.7	65.6	12.3

Linearity

One sample was diluted with assay buffer and tested. The results of Calprotectin concentration in the value of ng/mL are as follows:

DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
Neat	195.84	-	-
1:2	87.88	97.92	89.7
1:4	46.58	48.96	95.1
1:8	24.53	24.48	100.2
1:16	13.77	12.24	112.5

Spike Recovery

Three fecal extracts and three assay standards were spiked together in various volume combinations and tested. The results Calprotectin concentration in the value of ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	30.0	37.1	61.9	67.1	92.2
2	73.0	12.7	85.7	89.3	104.2
3	217.7	30.3	248.0	256.9	96.5

Additional ELISA Kits for IBD

- Fecal Myeloperoxidase (MPO) ELISA Kit, KT-880

- Fecal NGAL ELISA Kit, KT-853

Calprotectin, MPO and NGAL are three important proteins from neutriphils. However, their stability in vivo and in vitro are different.

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