

DKK-1 ELISA, Cat.No. BI-20413

For the quantitative determination of DKK-1 in human serum

ASSAY CHARACTERISTICS

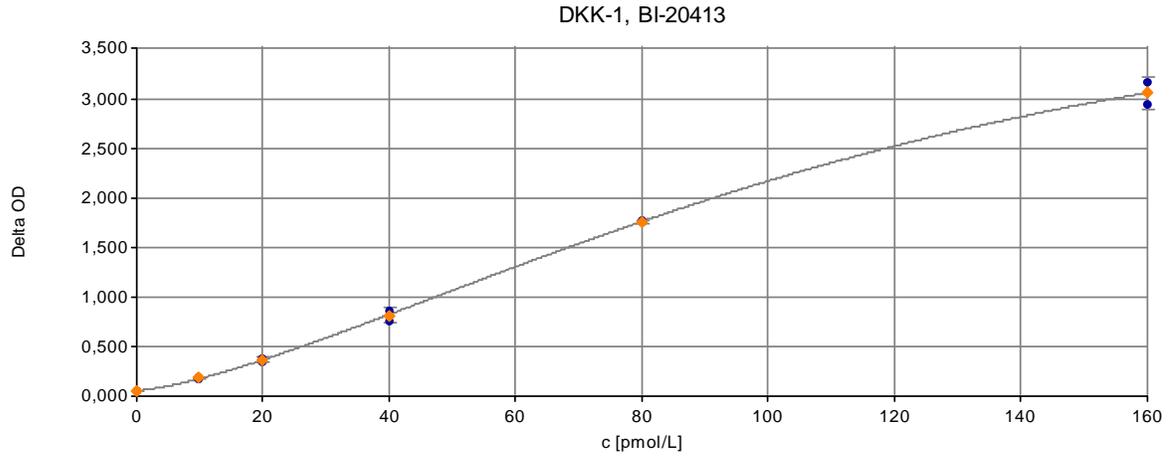
Method	Sandwich ELISA, 96-well strip plate, HRP/TMB			
Sample type	Serum			
Standard range	0-160 pmol/l (0, 10, 20, 40, 80, 160 pmol/l) (= 0-4103 pg/ml)			
Conversion factor	1 pg/ml = 0.039 pmol/l (MW = 25.8 kDa)			
Sample volume / well	20 µl human serum			
Incubation time, temp.	DAY TEST 2 h / 1 h / 30 min, room temperature (18-24°C)			
Sensitivity	LOD: 1.7 pmol/l (0 pmol/l + 3 SD) , LLOQ: 1.25 pmol/l			
Specificity	oligomeric forms of natural and recombinant human Dkk-1.			
Cross-reactivity	Human only, No cross-reactivity or interference with recombinant human Dkk-4, Kremen-1, Kremen-2 or LRP-6 is observed.			
Precision	Intra-assay (n=6) ≤ 3% , Inter-assay (n=9) ≤ 3%			
Spike/Recovery (average recovery spiked with 40 pmol/l rec. DKK-1)	Serum (n=8)= 92%			
Dilution linearity (average recovery of expected DKK-1)	Dilution:	1+1	1+3	1+7
	Serum (n=4)	109%	104%	100%
Values from apparently healthy individuals	Median Serum (n=51)= 34 pmol/l Each laboratory should establish its own reference range for the samples under investigation.			

Dilution linearity of endogenous DKK-1:

Dilution linearity of endogenous DKK-1 in human serum samples was tested by a 1+1, 1+3, and 1+7 dilution with STD1 (Standard 1, provided in the kit).

Sample matrix	Dilution	Mean recovery (%)	Range R [%]
Serum (n=4)	1+1	109	108-112
	1+3	104	99-107
	1+7	100	90-114

Typical standard curve:



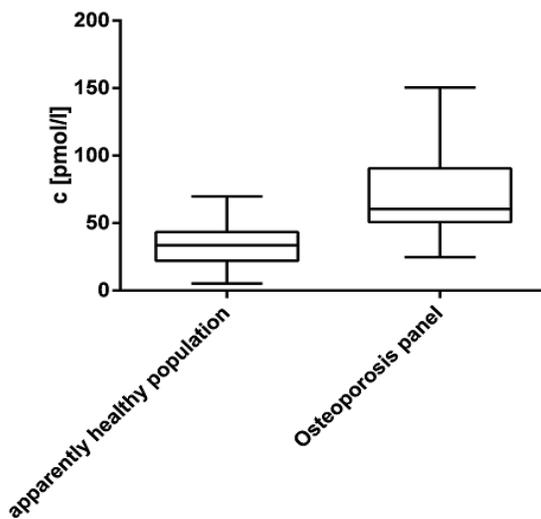
Values from apparently healthy individuals

Sample type	Serum (n=51)
Median (pmol/l)	34 (range 5-70)

It is recommended that each laboratory establishes its own reference range for the samples under investigation.

Values from a hospital panel (osteoporosis)

Sample type	Serum (n=48)
Median (pmol/l)	60 (range 25 - 150)



PERFORMANCE CHARACTERISTICS

Spike/Recovery of recombinant DKK-1:

Recovery of spiked human serum samples (n=8) was tested by adding different concentrations of recombinant DKK-1 (40 and 80 pmol/l) to human serum samples.

Sample	Spike (pmol/l)	Mean recovery (%)
Serum (n=8)	40	92
Serum (n=8)	80	83

Dilution linearity of endogenous DKK-1:

Dilution linearity of endogenous DKK-1 in human serum samples was tested by a 1+1, 1+3, and 1+7 dilution with STD1 (Standard 1, provided in the kit).

Sample matrix	Dilution	Mean recovery (%)	Range R [%]
Serum (n=4)	1+1	109	108-112
	1+3	104	99-107
	1+7	100	90-114

Dilution linearity of recombinant DKK-1:

Dilution linearity of recombinant DKK-1 in human serum matrix was tested by a 1+1, 1+3, 1+7 dilution with STD1 (Standard 1, provided in the kit).

Sample matrix	Dilution	Mean recovery (%)	Range R [%]
spiked serum (n=3)	1+1	106	91-116
	1+3	102	93-109
	1+7	102	100-105

Intra-assay precision & inter-assay precision:

Intra-assay: 2 samples of known concentrations were tested 5 times within 1 kit lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 9 times within 2 different kit lots and each in a different test assembly.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=9)	Sample 1	Sample 2
Mean (pmol/l)	19.9	80.1	Mean (pmol/l)	19.7	80.4
SD (pmol/l)	0.5	2.7	SD (pmol/l)	0.6	2.1
CV (%)	3	3	CV (%)	3	3

SAMPLE CHARACTERISTICS

The assay has been validated for the use of serum samples.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum. Allow samples to clot for 30 minutes at room temperature (RT) before performing serum separation by centrifugation, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C). Measure the acquired samples immediately or aliquot samples in polypropylene tubes and store at -25°C or lower. Avoid more than three freeze-thaw cycles.

Sample stability at RT, +4°C and -25°C:

6 human serum samples were aliquoted. The reference set was shock frozen after sample preparation. The second set was stored at RT (18-24°C) overnight. The recovery of calculated concentrations at RT is 91%.

Data show sample stability when stored overnight at RT:

Storage T	-25°C (ref)	RT overnight	
Sample ID	c (pmol/l)		R (%)
#1	10	8	87
#2	24	23	94
#3	6	6	96
#4	29	26	88
#5	35	35	98
#6	69	58	84
		Mean R (%)	91

Effect of freezing/thawing:

The mean recovery of DKK-1 concentration in human serum samples after 3 F/T cycles is 93%. Thus samples can be frozen at least 3 times.

Experiment:

Samples have been aliquoted, stored frozen and stressed by additional F/T cycles (frozen by storing them for 60 min at -25°C and the thawing process was at RT (18-24°C) for 60 min). The stressed samples were compared.

Data show F/T stress on samples by 3 and 6 F/T cycles:

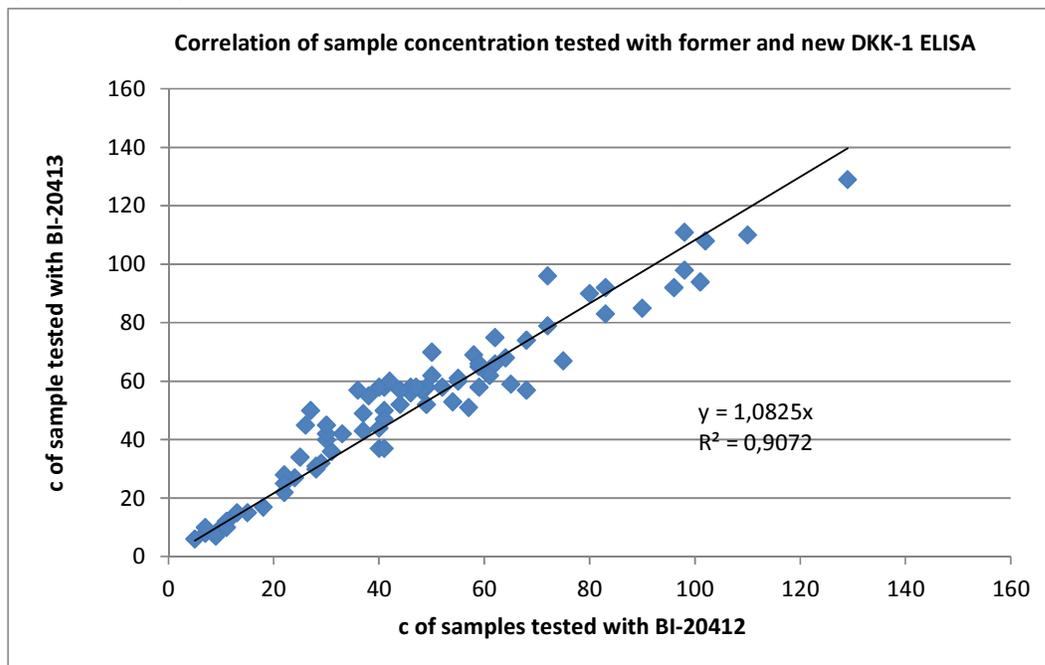
No. of F/T cycles	0 (ref)	3	6	3 F/T vs ref	6 F/T vs ref
Sample ID	c (pmol/l)			R (%)	R (%)
#S1	43	40	40	93	92
#S2	70	63	62	90	89
#S3	46	43	43	94	93
#S4	21	21	19	96	88
#S5	30	30	31	100	103
#S6	41	39	38	95	91
#S7	29	26	27	89	92
#S8	46	43	40	92	87
#S9	152	139	131	91	86
#S10	106	99	93	94	88
			Mean R (%)	93	91

Application on open ELISA instruments:

The assay was programmed on the ETI-Max3000. Standards and samples were tested. STDs and samples show a CV of <7% in the low to high calibration range. The coefficient of determination is >0.998 for a logit-log transformation. An assay protocol for the ETI-max3000 is available on request.

Comparison of DKK-1 concentrations in BI-20412 and BI-20413 (next generation DAY TEST):

DKK-1 values in the old (BI-20412) and new kit (BI-20413) versions were compared (n=78). The serum samples measured in both assay versions give comparable results (R² = 0.9072)



Rev: Jan 2015