

Supplementary figure 1



B. Specificity

Cross reactivity of the antibody used in the Free 25OH Vitamin D ELISA was determined by the supplier as depicted in the table.

Compound and Concentration	% Cross reaction
25OH-Vitamin D ₃ at 10 ng/mL	100
25OH-Vitamin D ₂ at 10 ng/mL	86
1, 25 (OH) ₂ -Vitamin D ₃ at 200 ng/mL	20
1, 25 (OH) ₂ -Vitamin D ₂ at 690 ng/mL	1.9
Vitamin D ₃ at 200 ng/mL	2.9
Vitamin D ₂ at 200 ng/mL	1.3
24, 25 (OH) ₂ -Vitamin D ₃ at 20 ng/mL	>100
25, 26 (OH) ₂ -Vitamin D ₃ at 4 ng/mL	>100
3 epi 25OH-Vitamin D ₃ at 20 µg/mL	0.1

C. Intermediate Precision & Repeatability

Intermediate Precision and repeatability is determined based on CLSI EP05-A3.

Sample	N	pg/mL		Repeatability (within run)	Intermediate Precision (Total)
Pool 1	80	5.8	SD	0.29	0.34
			CV	4.9%	5.9%
Pool 2	80	9.6	SD	0.53	0.59
			CV	5.5%	6.1%
Pool 3	80	18.4	SD	0.35	0.75
			CV	1.9%	4.0%
Pool 4	80	28.1	SD	1.26	1.76
			CV	4.5%	6.3%