

18 ASSAY PROCEDURE

Preparation of reagents		Reconstitution:	Dilution
A-G	Standards	in 500 µL Dilution Buffer VP	-
KS1	Control Serum 1	in 250 µL Dilution Buffer VP	≥ 1:16 with VP
KS2	Control Serum 2	in 250 µL Dilution Buffer VP	≥ 1:16 with VP
WP	Washing Buffer	-	1:20 with Aqua dest
Sample dilution: with Dilution Buffer VP ≥ 1:16			
Before assay procedure bring all reagents to room temperature 20-25°C .			
Assay Procedure in Double Determination:			
Pipette	Reagents	Position	
50 µL	Antibody Conjugate AK	Pipette in <u>all</u> required number of wells	
50 µL	Standard A (0 ng/mL)	A1/A2	
50 µL	Standard B (0.1 ng/mL)	B1/B2	
50 µL	Standard C (0.5 ng/mL)	C1/C2	
50 µL	Standard D (1 ng/mL)	D1/D2	
50 µL	Standard E (2 ng/mL)	E1/E2	
50 µL	Standard F (4 ng/mL)	F1/F2	
50 µL	Standard G (8 ng/mL)	G1/G2	
50 µL	Control Serum KS 1 (≥ 1:16 diluted)	H1/G2	
50 µL	Control Serum KS 2 (≥ 1:16 diluted)	A3/A4	
50 µL	Sample (≥ 1:16 diluted)	in the rest of the wells according the requirements	
Cover the wells with the sealing tape.			
Sample Incubation with Shaking: 1 h at 20°C - 25°C, 350 rpm			
5 x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well	In each well	
100 µL	Enzyme Conjugate EK	In each well	
Cover the wells with the sealing tape.			
Incubation with Shaking: 30 Minutes at 20°C - 25°C, 350 rpm			
5 x 300 µL	Aspirate the contents of the wells and wash 5 x with 300 µL each Washing Buffer WP/ well	In each well	
100 µL	Substrate Solution S	In each well	
Incubation: 15 Minutes in the Dark at 20°C - 25°C			
100 µL	Stopping Solution SL	In each well	
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.			