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 all 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.		