## 16 ASSAY PROCEDURE

| **Preparation of reagents** | | |
|---|---|---|
| **A-E**<br>**KS1**<br>**KS2**<br>**AK**<br>**EK** | **Standards** (each 750 µL)<br>**Control Serum 1** (750 µL)<br>**Control Serum 2** (750 µL)<br>**Antibody Conjugate** (12 mL)<br>**Enzyme Conjugate** (12 mL) | Bring the ready for use reagents to room temperature: **20°-25°C** |
| **WP** | **Washing Buffer** | Dilute **1:20** with **Aqua dest.** |
| **Dilution of samples is generally not necessary;** just use **20 µL** per single determination. | | |
| Before assay procedure bring all reagents to room temperature **20-25°C**. | | |
| **Assay Procedure in Double Determination:** | | |
| **Pipette** | **Reagents** | **Position** |
| 100 µL | Dilution Buffer **VP** | Pipette in all **required number of wells** |
| 20 µL | Dilution Buffer **VP** (**Blank**) | A1/A2 |
| 20 µL | Standard **A** (**1 ng/mL**) | B1/B2 |
| 20 µL | Standard **B** (**5 ng/mL**) | C1/C2 |
| 20 µL | Standard **C** (**10 ng/mL**) | D1/D2 |
| 20 µL | Standard **D** (**15 ng/mL**) | E1/E2 |
| 20 µL | Standard **E** (**25 ng/mL**) | F1/F2 |
| 20 µL | Control Serum **KS1** | G1/G2 |
| 20 µL | Control Serum **KS2** | H1/H2 |
| 20 µL | Sample | Pipette sample in the rest of the wells according the requirements (**Pipetting Control** = colour turns red!) |
| Cover the wells with the sealing tape. | | |
| **Sample Incubation**: **1 h at 20-25°C, 350 rpm** | | |
| 3 x 300 µL | Aspirate the contents of the wells and **wash** 3 x with **300 µL** each Washing Buffer **WP/ well** | In each well |
| 100 µL | Antibody Conjugate **AK** | In each well |
| Cover the wells with the sealing tape. | | |
| **Incubation: 30 Minutes at 20-25°C, 350 rpm** | | |
| 100 µL | Enzyme Conjugate **EK, without washing the wells (!)** – add to the previously pipetted Antibody Conjugate **AK**-solution **thereto** mix shortly through cautious tapping on the side of the **MTP**. **Attention:** high filled volume of the wells! | In each well |
| Cover the wells with the sealing tape. | | |
| **Incubation: 30 Minutes at 20-25°C, without shaking** | | |
| 3 x 300 µL | Aspirate the contents of the wells and **wash 3 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 20-25°C** | | |
| 100 µL | Stopping Solution **SL** | In each well |
| Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength. | | |