

Summary of DF1 Immunoassay Protocol

1. Prepare the samples according to the appropriate Application Note.
2. Place the anti-Dioxin **antibody coated tubes** in Grip-Rack and label.
3. Prerinse tubes once by **filling** each tube with **reagent grade or bottled distilled water**. Dump water out and tap inverted tubes on absorbent material to remove excess water.
4. Add **0.5 mL of Sample Diluent** to each tube using Repeater Pipettor.
5. Add **50 µL of standard (0, 0.064, 0.2, 0.64, and 2.0 ppb 2378-TCDD) or 50 µL of prepared sample directly to liquid** in each tube. Mix each tube immediately after addition and let stand 2 hours (acceptable performance) or **overnight (12 to 24 hours; best performance)**.
6. Remove tube contents and **wash 4 times** using **reagent grade or bottled distilled water plus 0.01% Triton X-100, 1.0 mL** per tube for each wash. Tap inverted tubes on absorbent material to remove excess water.
7. Add **0.5 mL of Competitor-HRP Conjugate** to each tube using Repeater Pipettor. Let stand **15 minutes**.
8. Remove tube contents and **wash 4 times** using **1.0 mL reagent grade or bottled distilled water** per tube for each wash. Tap inverted tubes on absorbent material to remove excess water.
9. Add **0.5 mL of HRP Substrate** to each tube using Repeater Pipettor. Let stand **30 minutes**.
10. Add **0.5 mL of Stop** to each tube using Repeater Pipettor. **Wipe** outside dry and **read OD of entire batch immediately**.