**8-Isoprostane EIA Short Protocol**

1. **EIA Buffer** - Dilute with 90 ml of UltraPure water.

2. **Wash Buffer** - Dilute the 5 ml vial to 2 L and add 1 ml Polysorbate 20 or dilute the 12.5 ml vial to 5 L and add 2.5 ml Polysorbate 20.

3. **Tracer** - Reconstitute the 100 dtw vial with 6 ml of EIA Buffer or the 500 dtw vial with 30 ml EIA Buffer.

4. **Antibody** - Reconstitute the 100 dtw vial with 6 ml of EIA Buffer or the 500 dtw vial with 30 ml EIA Buffer.

5. **Standard** - Prepare as described in the figure below.

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**Steps** | **Reagent** | **Blank** | **TA** | **NSB** | **B₀** | **Std/Sample**
---|---|---|---|---|---|---
1. Add Reagents | EIA Buffer | -- | -- | 100 μl | 50 μl | --
 | Standard/Sample | -- | -- | -- | -- | 50 μl
 | Tracer | -- | 5 μl (at devl. step) | 50 μl | 50 μl | 50 μl
 | Antiserum | -- | -- | -- | 50 μl | 50 μl
2. Incubate | Cover plate and incubate 18 hours at 4°C
3. Wash | Wash all wells five times
4. Add Reagents | Tracer | -- | 5 μl | -- | -- | --
 | Ellman’s | 200 μl | 200 μl | 200 μl | 200 μl | 200 μl
5. Incubate | Cover plate and incubate 90-120 minutes RT in the dark
6. Read | Read plate at a wavelength between 405-420 nm