### Testosterone 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 dtn vial with 6 ml of EIA Buffer or the 500 dtn vial with 30 ml EIA Buffer.
4. **Antibody** - Reconstitute the 100 dtn vial with 6 ml of EIA Buffer or the 500 dtn vial with 30 ml EIA Buffer.
5. **Standard** - Prepare as described in the figure below.

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**Steps** | **Reagent** | **Blank** | **TA** | **NSB** | **B<sub>0</sub>** | **Std/Sample**
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1. Add Reagents | EIA Buffer | -- | -- | 100 μl | 50 μl | --
Standard/Sample | -- | -- | -- | -- | -- | 50 μl
Tracer | -- | -- | 50 μl | 50 μl | 50 μl | 50 μl
Antibody | -- | -- | -- | 50 μl | 50 μl | 50 μl

2. Incubate - Cover plate and incubate two hours at room temperature (22°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 60-90 minutes RT with gentle shaking

6. Read - Read plate at a wavelength between 405-420 nm