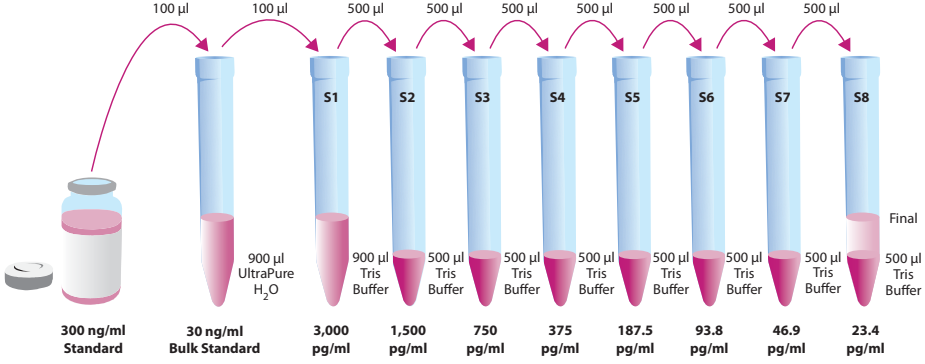
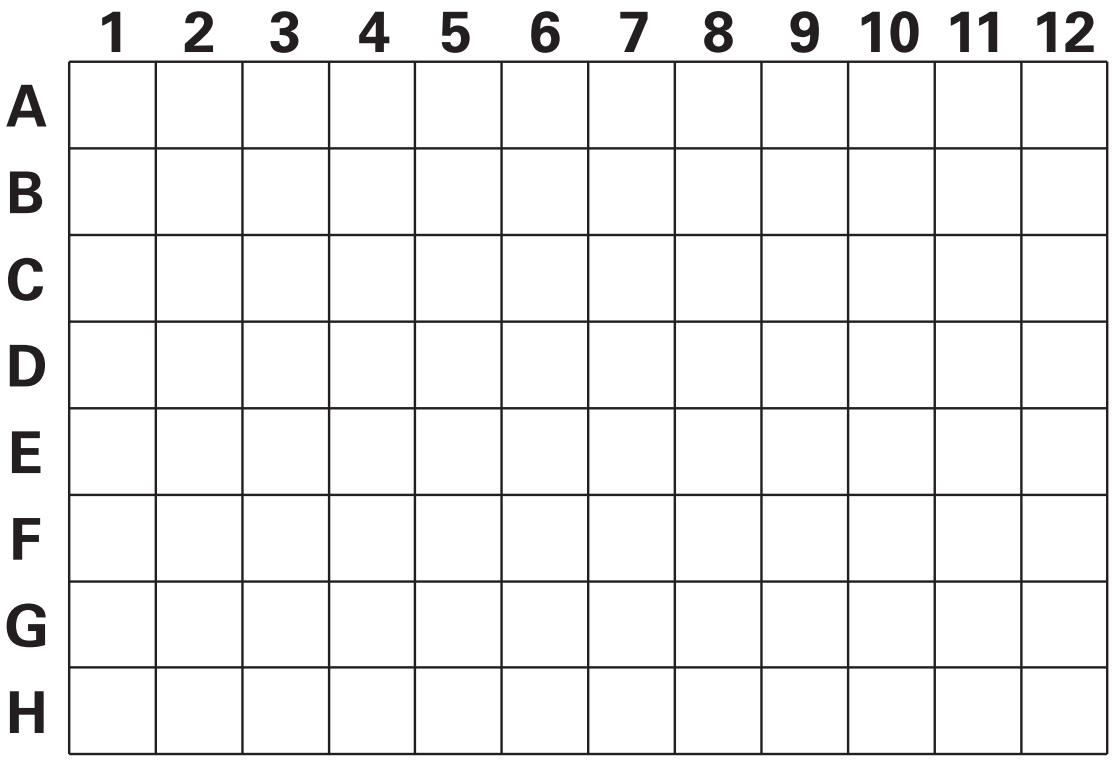
STAT-8-Isoprostane ELISA Short Protocol

1. **Tris Buffer** - Dilute to 100 ml with UltraPure water.
2. **AP Wash Buffer** - Dilute the 5 ml vial to 750 ml or dilute the 12.5 ml vial to 1,875 ml.
3. **DEA Buffer** - Dilute the 2.5 ml vial to 25 ml or dilute the 12.5 ml vial to 125 ml.
4. **Tracer** - Reconstitute the 100 dtn vial with 6 ml of Tris buffer or the 500 dtn vial with 30 ml Tris buffer.
5. **Antiserum** - Reconsitute the 100 dtn vial with 6 ml of Tris buffer or the 500 dtn vial with 30 ml Tris buffer.
5. **Standard** - Prepare as described in the figure below.



Steps	Reagent	Blank	TA	NSB	B_0	Std/Sample
1. Add Reagents	Tris Buffer	--	--	100 µl	50 µl	--
	Standard/Sample	--	--	--	--	50 µl
	Tracer	--	--	50 µl	50 µl	50 µl
	Antiserum	--	--	--	50 µl	50 µl
2. Incubate	Cover plate and incubate one hour at room temperature (22°C) on an orbital shaker					
3. Wash	Wash all wells five times					
4. Add Reagents	Tracer	--	5 µl	--	--	--
	*p*NPP	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					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