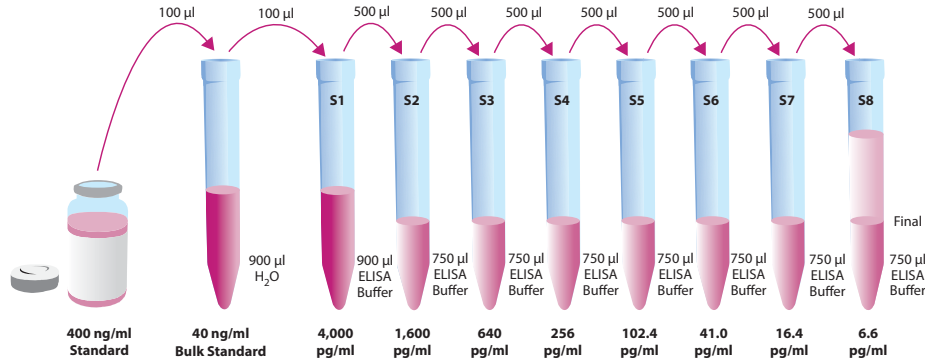


Estradiol ELISA Short Protocol

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



Steps	Reagent	Blank	TA	NSB	B ₀	Std/Sample
1. Add Reagents	ELISA Buffer	--	--	100 µl	50 µl	--
	Standard/Sample	--	--	--	--	50 µl
	Tracer	--	--	50 µl	50 µl	50 µl
	Antibody	--	--	--	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	--	--	--
	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					

[illegible]