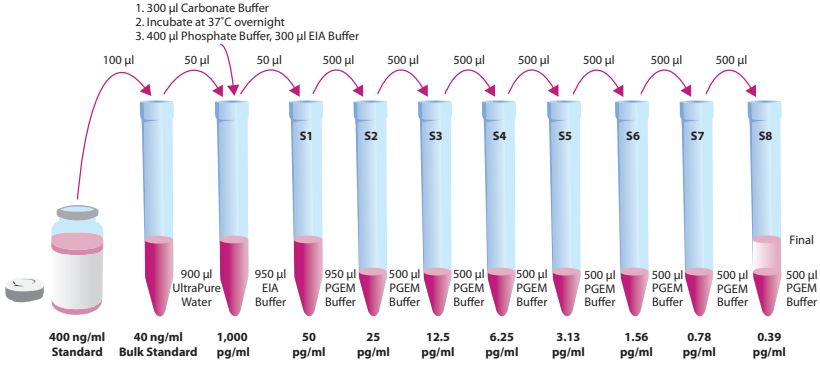
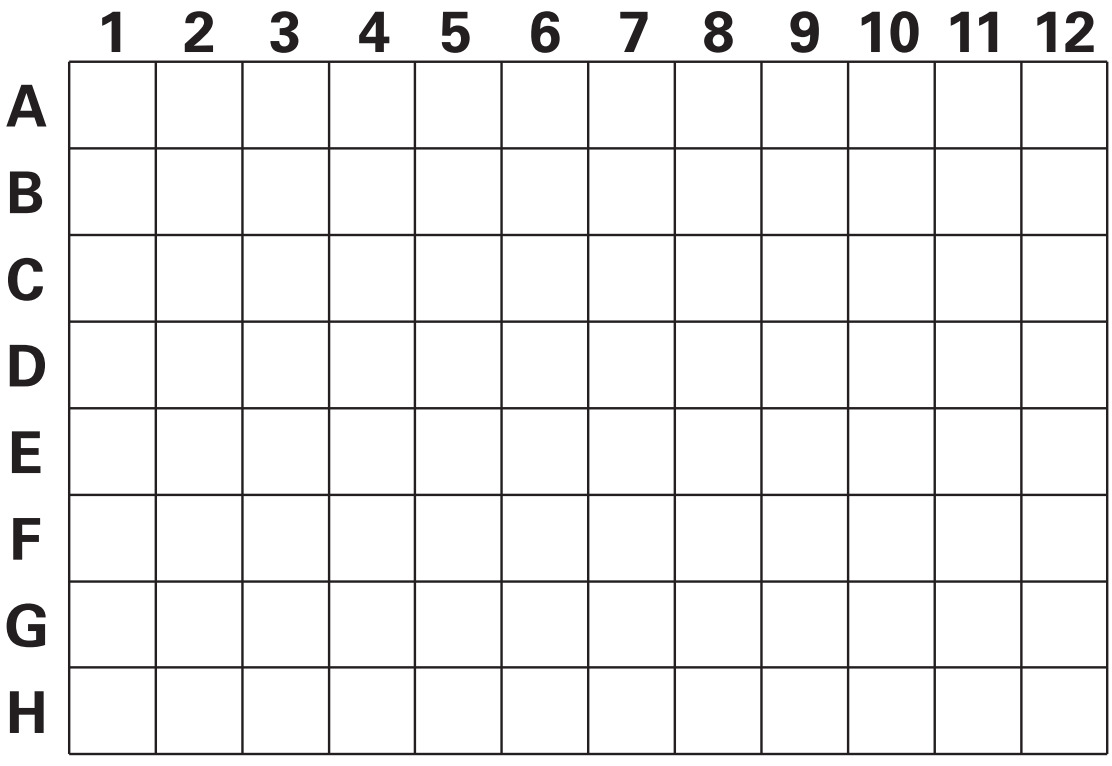
Prostaglandin E Metabolite EIA Short Protocol

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 Tween 20 or dilute the 12.5 ml vial to 5 L and add 2.5 ml Tween 20.
3. **PGEM Assay Buffer** - Prepare 20 ml of PGEM Assay Buffer by combining 13 ml EIA Buffer, 3 ml Carbonate Buffer*, and 4 ml Phosphate Buffer*. (*See booklet for details.)
4. **Tracer** - Reconstitute the 100 dtn vial with 6 ml of EIA Buffer or the 500 dtn vial with 30 ml EIA Buffer.
5. **Antiserum** - Reconsitute the 100 dtn vial with 6 ml of EIA Buffer or the 500 dtn vial with 30 ml EIA Buffer.
6. **Standard** - Prepare as described in the figure below.



Steps	Reagent	Blank	TA	NSB	B_0	Std/Sample
1. Add Reagents	EIA Buffer	--	--	50 µl	--	--
	Standard/Sample	--	--	--	--	50 µl
	Tracer	--	--	50 µl	50 µl	50 µl
	Antiserum	--	--	--	50 µl	50 µl
	PGEM Buffer	--	--	50 µl	50 µl	--
2. Incubate	**Cover plate and incubate 18 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**					


1 2 3 4 5 6 7 8 9 10 11 12
A
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