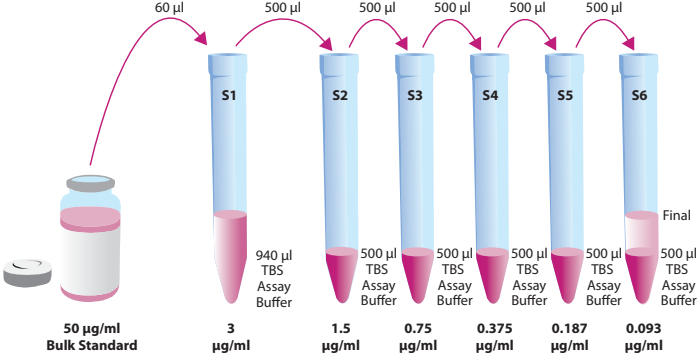
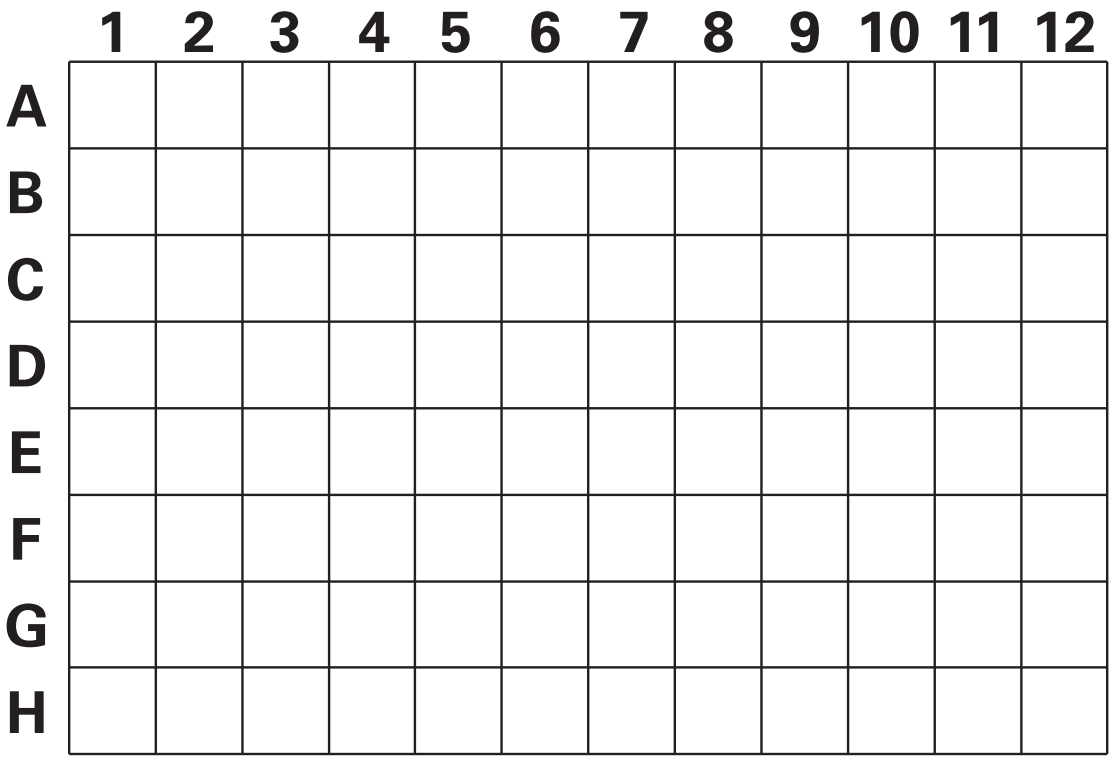
His-Express Detection EIA Short Protocol

1. **TBS Buffer** - Dilute with 90 ml of UltraPure water.
2. **DEA Buffer** - Dilute the 2.5 ml vial to 25 ml or dilute the 12.5 ml vial to 125 ml of UltraPure water.
3. **AP Buffer** - Dilute the 5 ml vial to 750 ml or dilute the 12.5 ml vial to 1,875 ml of UltraPure water.
4. **Tracer** - Reconstitute the 100 dtn vial with 6 ml of TBS Buffer or the 500 dtn vial with 30 ml TBS Buffer.
5. **Antibody** - Reconsitute the 100 dtn vial with 6 ml of TBS Buffer or the 500 dtn vial with 30 ml TBS Buffer.
6. **Standard** - Prepare as described in the figure below.



Steps	Reagent	Blank	NSB	B_0	Std/Sample
1. Add Reagents	TBS 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 90 minutes at room temperature (22°C) on an orbital shaker**				
3. Wash	**Wash all wells four times**				
4. Add Reagents	*p*NPP Substrate Solution	200 µl	200 µl	200 µl	200 µl
5. Incubate	**Cover plate and incubate 30-60 minutes at room temperature**				
6. Read	**Read plate at a wavelength between 405-420 nm**				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