



8. *Optional:* Pipette 50µl Gd-DTPA standards into each well as designated in your plate layout.
9. *Optional:* Vortex Gd-DTPA Controls to mix. Pipette 50µl Controls as designated by your plate layout.
10. Pipette 50µl diluted experimental samples as designated by your plate layout.
11. Pipette 50µl HRP-Gadolinium DTPA (**yellow**) into **all wells (except Blanks)**. Tap plate gently to mix contents of each well and observe wells for consistency.
12. Pipette 50µl Rabbit anti-Gadolinium DTPA (**blue**) into **all wells (including Blanks)**. Tap plate gently to mix contents of each well and observe wells for consistency. Peel paper backing from Plate Sealer and carefully place Plate Sealer (sticky side down) over plate. Use paper backing to cover plate and protect wells from light.
13. Place plate on Plate Shaker set to 500 rpm and time for 90 minutes.
14. When plate incubation from Step 12 is complete, wash plate three times with 350µl Wash Buffer per well.
15. Check HRP Substrate Reagent to be sure it is colorless. Do not use if blue (contaminated). Pipette 100µl into **all wells (including Blanks)**. Tap gently to mix contents of each well.
16. Incubate substrate for 30 minutes at room temperature (no shaking). Before end of 30-minute incubation, look over plate for any bad wells and circle on your plate layout.
17. Pipette 100µl of HRP Stop Reagent into **all wells (including Blanks)**. Tap gently to mix contents of each well.
18. Read plate at 450nm and use 4-parameter curve-fit for data reduction. Remember to account for the dilution of samples.

19.

**SCHEME 1: REPRESENTATIVE PLATE LAYOUT**

Standard Curve  
& Controls

|-----Subject serum samples: one color per Subject-----|

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	B	B										
<b>B</b>												
<b>C</b>												
<b>D</b>												
<b>E</b>												
<b>F</b>												
<b>G</b>												
<b>H</b>												

**Notes:**

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## Appendix 1: Detailed instructions on how to make a Standard Curve

1. Refer to Figure 1 for schematic. Set up five glass culture tubes in a row, leave a space, then three more glass culture tubes in a row.
2. Label the first five tubes with the following concentrations of Gadolinium-DTPA (in  $\mu\text{g/ml}$ ): 100, 10, 1, 0.1, 0.01. Then label the last three tubes: 0.3, 0.03, and 0.003.
3. Mark 0.003 with **B**, 0.01 with **C**, 0.03 with **D**, 0.1 with **E**, and 0.3 with **F**. These letters correspond to the row each of the five Standards falls on in the Representative Plate Layout (Scheme 1). Although each individual researcher's plate configuration may change, it is strongly recommended the positioning of the standard curve remain in the first three columns of the 96-well plate.
4. Add 900 $\mu\text{l}$  Diluent to every tube except tube labeled "0.3 / F." Add 970 $\mu\text{l}$  Diluent to tube labeled "0.3/F."
5. Vortex microtube containing Gadolinium-DTPA Concentrate (1000 $\mu\text{g/ml}$ ), Catalog # FIT-0103. Withdraw 100 $\mu\text{l}$  of Gadolinium-DTPA Concentrate from microtube and add to tube labeled "100" and vortex to mix.
6. Withdraw 100 $\mu\text{l}$  from tube labeled "100" and add to tube labeled "10" and vortex to mix.
7. Withdraw 100 $\mu\text{l}$  from tube labeled "10" and add to tube labeled "1" and vortex to mix.
8. Withdraw 100 $\mu\text{l}$  from tube labeled "1" and add to tube labeled "0.1" and vortex to mix.
9. Withdraw 100 $\mu\text{l}$  from tube labeled "0.1" and add to tube labeled "0.01" and vortex to mix.
10. Withdraw 30 $\mu\text{l}$  from tube labeled "10" and add to tube labeled "0.3" and vortex to mix.
11. Withdraw 100 $\mu\text{l}$  from tube labeled "0.3" and add to tube labeled "0.03" and vortex to mix.
12. Withdraw 100 $\mu\text{l}$  from tube labeled "0.03" and add to tube labeled "0.003" and vortex to mix.
13. Transfer 850 $\mu\text{l}$  of all dilutions marked with **B-F** into the first 5 positions of a string of 8 microtubes, placing concentrations in ascending order:
  - 1<sup>st</sup> microtube: 850 $\mu\text{l}$  "B/ 0.003"
  - 2<sup>nd</sup> microtube: 850 $\mu\text{l}$  "C/ 0.01"
  - 3<sup>rd</sup> microtube: 850 $\mu\text{l}$  "D/0.03"
  - 4<sup>th</sup> microtube: 850 $\mu\text{l}$  "E/0.1"
  - 5<sup>th</sup> microtube: 850 $\mu\text{l}$  "F/0.3"
14. Standard curve is now prepared and is ready to be used in assay.

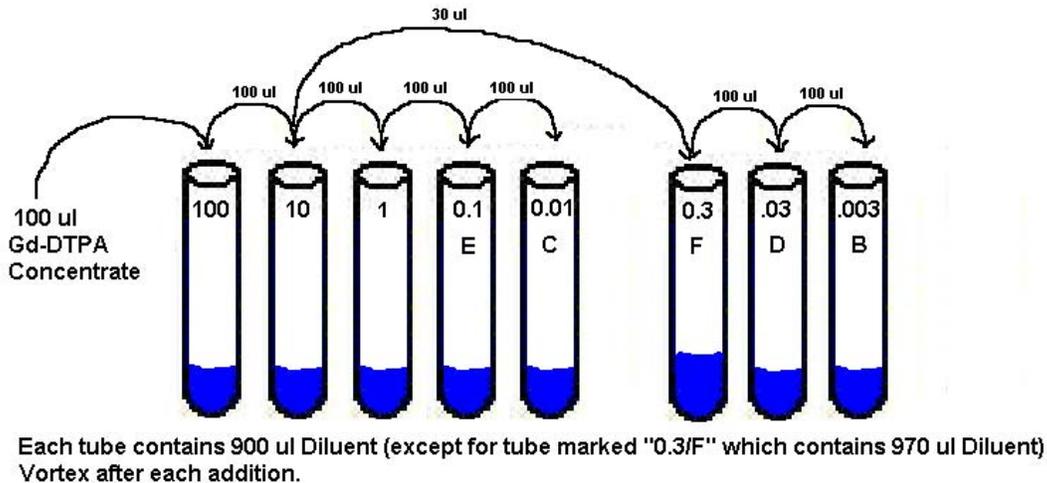


Figure 1: Schematic representation of Standard Curve preparation