

### *Producing the Standard Curve*

The standard curve is a serial dilution consisting of known concentrations of testosterone (or other hormone). Your samples are compared to this curve to determine the unknown concentrations.

Serially dilute the testosterone standard as follows:

- First label nine 13 X 100 test tubes #1 - #9. If you are running 1 or 2 plates use the following protocol:
- Add 1 ml of Assay Buffer 3 to tube #1 and 500  $\mu$ l of Assay Buffer 3 to tubes #2 - #9.
- Remove 40  $\mu$ l of buffer from tube #1.
- Add 40 $\mu$ l of the testosterone standard supplied with the kit (50,000 pg/ml) to tube #1. Vortex thoroughly.
- Add 500  $\mu$ l of tube #1 to tube #2 and vortex thoroughly.
- Add 500  $\mu$ l of tube #2 to tube #3 and vortex.
- Continue this for tubes #4 - #9. These diluted standards should be used within 60 min of preparation.

The concentrations of testosterone in tubes #1 through #9 will be 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 pg/ml, respectively.

Because you will add 100  $\mu$ l of each standard to duplicate wells on the EIA plate, the concentrations of the standards in the standard curve can also be expressed as 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 pg/well. Expressing standard concentrations in this form will make assay calculations easier.

If you are running 3 or 4 plates, double all of the volumes listed above in order to prepare sufficient amounts of each standard.