

**Eicosanoid Primary Standards**  
**8/27/2005**  
**LIMS Protocol # PS0000000603**

We are currently using these primary standards: PGF<sub>2</sub>α, PGE<sub>2</sub>, PGD<sub>2</sub>, PGJ<sub>2</sub>, 15 deoxy PGD<sub>2</sub>, 15 deoxy PGJ<sub>2</sub>, 11 HETE, 5 HETE, and AA. These standards are obtained from Cayman with accurately determined quantities in 1 mg amounts. Stock solutions were made of each of these by dissolving the compound in the same solvent that Cayman uses to ship the given compound. All samples were diluted to give a stock solution of about 500 ng/μl. The exact concentration was either calculated from the weight given by Cayman or by uv absorbance when this was available. The exact concentrations are used in the calculations in place of the 500 ng/ul value.

Compound	Cayman Cat #	Nominal Stock Conc.
PGF <sub>2</sub>	16010	500 ng/ μl
PGE <sub>2</sub>	14010	500 ng/ μl
PGD <sub>2</sub>	12010	500 ng/ μl
PGJ <sub>2</sub>	18500	500 ng/ul
15 deoxy PGD <sub>2</sub>	12700	500 ng/ul
15 deoxy PGJ <sub>2</sub>	18570	500 ng/ul
11 HETE	34510	500 ng/ul
5 HETE	34230	500 ng/ul
AA	90010	500 ng/ μl

These samples are given a reagent barcode.

From each of these stocks, a 50 ng/μl stock is made (100 ul of stock plus 900 ul ethanol). This solution is given a solution barcode. These stocks are used to analyze and follow the purity of the standards with time.

A working stock is then made that contains 2.5 ng/ul of each standard (100 ul of each stock plus 1100 ul ethanol). Then appropriate dilutions are made to achieve the Primary Stocks listed below; again diluted in ethanol. These solutions are then used to make the primary standards on the day that they will be used via the following protocol using the 0.1 ng/ul internal standard prepared via protocol PS0000000501:

Final Primary ng	Final Internal STD ng	Primary STD ul	Primary Stock ng/ul	Int STD 100
0.3	10	40	0.0075	100
1	10	40	0.025	100
3	10	40	0.075	100
10	10	40	0.25	100
30	10	40	0.75	100
100	10	40	2.5	100

The Final values are ng not concentration since the volumes vary; however, only the ratio of primary to internal std is important.

Each set of daily standards are given a single new solution barcode.