
Aromatase Optimization Supplementary Studies – Experiment #1

Draft Report

November 6, 2003

Study Objectives

To standardize the protocol for the $^3\text{H}_2\text{O}$ aromatase assay using human placenta microsomes in the drug metabolism laboratories at RTI using appropriate controls plus a comparison with data from the laboratory of Dr. Robert Brueggemeier's lab. This is the first of three supplemental experiments to ensure that the placental aromatase assay is optimized and standardized before beginning the multiple chemical comparison study. The degree of inhibition of the reaction by 4-hydroxyandrostenedione (4-OH-ASDN) was investigated to demonstrate the mediation of the reaction by aromatase.

Study Results

Replicate 1

The aromatase assay was conducted as described in the protocol using human placental microsomes at three final protein concentrations (0.05, 0.025 and 0.0125 mg/mL). Each condition was performed in triplicate. Four conditions (for each protein concentration) were tested:

1. Complete system (microsomes, substrate, complete NADPH regenerating system)
2. Inhibited system (microsomes, substrate, complete NADPH regenerating system, 4-OH-ASDN)
3. Blank (boiled microsomes, substrate, NADPH regenerating system minus NADP)
4. No NADP (microsomes, substrate, NADPH regenerating system minus NADP)

The specific activity of the stock ^3H ASDN was 25.3 Ci/mmol. ^3H ASDN was combined with nonradiolabeled ASDN to prepare the substrate solution for use in the assays and the final specific activity of ^3H ASDN in the substrate solution was 1.68 $\mu\text{Ci}/\mu\text{g}$.

The spreadsheets for this replicate are presented as Tables 1-3. The two higher protein concentrations yielded substrate conversion percentages (38 and 18% for 0.05 and 0.025 mg/mL, respectively) in excess of the target (10-15%). The lowest protein concentration (0.0125 mg/mL) yielded ca. 9% conversion of substrate to product in 15 min. These substrate conversion percentages are in excellent agreement with those (36.8, 19.6 and 8.1% for 0.05, 0.025 and 0.0125 mg/mL, respectively) predicted in the analysis of the previously reported optimization data by Dr. Paul Feder. Variance within each triplicate set of tubes was low, with

the coefficient of variance of aromatase activity less than 3% in both the complete and inhibited systems.

The ‘No NADP’ tubes show considerable activity, possibly because of residual NADP in the microsomal preparation or from cross-contamination of these tubes with NADP (during the pipetting process). Because the activity in those tubes was so high, the data from the ‘Blank’ tubes was used as background when calculating aromatase activity in the ‘Complete’ and ‘Inhibited’ tubes.

The results for the ‘Complete’ and ‘Inhibited’ tubes are presented in Table 4 and graphically in Figure 1.

Aromatase product formation increased linearly with increasing protein concentration. Total aromatase activity was fairly constant at 0.0501 ± 0.0016 nmol/mg/min over the range of protein concentrations tested. 4-OH-ASDN (100 nM) inhibited aromatase activity by about 76% at each protein concentration.

Follow-on Experiment

An additional experiment was performed in order to address the question of whether the activity found in the ‘No NADP’ tubes may be due to residual NADP in the microsomes. The aromatase assay was conducted as described in the protocol using three final protein concentrations (0.05, 0.025 and 0.0125 mg/mL). Each condition was performed in triplicate. Three conditions (for each protein concentration) were tested:

1. Complete system (microsomes, substrate, complete NADPH regenerating system)
2. No NADP (microsomes, substrate, NADPH regenerating system minus NADP)
3. No NADPH regenerating system (microsomes, substrate, no NADPH regenerating system).

For this experiment, the final substrate specific activity was 1.56 $\mu\text{Ci}/\mu\text{g}$.

The spreadsheets for this experiment are presented as Tables 5-7. The two higher protein concentrations yielded substrate conversion percentages (45 and 21% for 0.05 and 0.025 mg/mL, respectively) in excess of the target 10-15%. The lowest protein concentration (0.0125 mg/mL) yielded ca. 10% conversion of substrate to product in 15 min.

The ‘No NADP’ tubes again showed measurable activity but that activity was much lower than was found in the first replicate. The ‘No regenerating system’ tubes gave essentially no turnover of substrate. The radioactive content of the aqueous portion of these tubes was very similar to that found in the ‘Blank’ (boiled microsomes) tubes from the first replicate and represented only about 0.1% of the radioactivity in the reaction.

Product formation was again linear with protein concentration (Table 8). Aromatase activity was slightly higher than in the first replicate (0.0573 ± 0.0026 nmol/mg/min vs. 0.0501 ± 0.0016 nmol/mg/min, respectively).

Replicate 2

The aromatase assay was conducted as described in the protocol using human placental microsomes at three final protein concentrations (0.05, 0.025 and 0.0125 mg/mL). Each condition was performed in triplicate. Six conditions (for each protein concentration) were tested:

1. Complete system (microsomes, substrate, complete NADPH regenerating system)
2. Inhibited system (microsomes, substrate, complete NADPH regenerating system, 4-OH-ASDN)
3. Blank (boiled microsomes, substrate, NADPH regenerating system minus NADP)
4. No NADP (microsomes, substrate, NADPH regenerating system minus NADP)
5. No NADPH regenerating system (microsomes, substrate, no NADPH regenerating system)
6. NADPH (microsomes, substrate, 0.3 mM NADPH [instead of the regenerating system])

For this experiment, the final substrate specific activity was $2.30 \mu\text{Ci}/\mu\text{g}$.

The spreadsheets for this experiment are presented as Tables 9-11. The two higher protein concentrations yielded substrate conversion percentages (45 and 21% for 0.05 and 0.025 mg/mL, respectively) in excess of the target (10-15%). The lowest protein concentration (0.0125 mg/mL) yielded ca. 10% conversion of substrate to product in 15 min.

The ‘No NADP’ tubes showed similar activity to that found in the follow-on experiment above. The aqueous portions of the ‘No regenerating system’ and the ‘Blank’ (boiled microsomes) tubes again contained very minimal radioactivity (about 0.1% of the total). The activity found in the NADPH tubes was similar to that found in the ‘Complete’ tubes of this set.

Product formation was again linear with protein concentration (Table 12 and Figure 2). Aromatase activity was similar to that found in the follow-on experiment (0.0567 ± 0.0030 nmol/mg/min vs. 0.0573 ± 0.0026 nmol/mg/min, respectively).

Summary and Conclusions

Product formation rates (nmol/min), substrate conversion and percent inhibition data for all three experiments are presented in Table 13. Day-to-day variance in the product formation rate was low (ca. 7.4%). Only the 0.0125 mg/mL reaction set had less than 15% substrate conversion under the reaction conditions.

Aromatase activity data for all three experiments is presented in Table 14. In each case, the radioactivity in the Blank tubes was subtracted from that in the assay tubes prior to calculation of aromatase activity. The calculated activity in the No NADP tubes of the follow-on experiment and replicate 2 sets was much lower than that found for the first replicate. A change in pipetting order was initiated between the first replicate and the follow-on experiment that was intended to minimize the possibility of NADP carryover from tube to tube. This change may explain the widely varying activities between the first replicate and the follow-on ‘No NADP’ tubes; the high apparent activity in the first replicate ‘No NADP’ tubes may be due to their inadvertent contamination with NADP. Only about 0.1% of the radioactivity in the reaction mixture remained in the aqueous phase of the ‘Blank’ and ‘No system’ tubes after extraction. The use of 0.3 mM NADPH instead of the regenerating system gave aromatase activities similar to those found in the tubes containing the regenerating system. At a protein concentration of 0.0125 mg/mL, the aqueous portion contained approximately 15000 DPM/mL after extraction. If the activity were inhibited 90% by a test substance, the aqueous portion would be expected to contain about 1500 DPM/mL. This is still an easily quantifiable level of radioactivity. Therefore, a protein level of 0.0125 mg/mL is suggested for use in the next studies.

Future Work

A sample of our human placenta microsomes has been shipped to Dr. Brueggemeier. Upon receipt of his data (obtained using that microsomal preparation), we will compare our data with that reported from his laboratory.

Table 1A. Replicate 1: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info		Calculate DPM in aqueous portion after extraction								Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.05	Complete	1	2	0.5	1	20611	41222	41232	82464	0.1	218472	37.75	82241	0.0768
				0.5	2	20621	41242			0.1				
		2	2	0.5	1	20948	41896	42545	85090	0.1	218472	38.95	84867	0.0793
				0.5	2	21597	43194			0.1				
		3	2	0.5	1	20787	41574	41718	83436	0.1	218472	38.19	83213	0.0777
				0.5	2	20931	41862			0.1				
	Inhibition	1	2	0.5	1	5012	10024	10004	20008	0.1	218472	9.16	19785	0.0185
				0.5	2	4992	9984			0.1				
		2	2	0.5	1	5166	10332	10379	20758	0.1	218472	9.50	20535	0.0192
				0.5	2	5213	10426			0.1				
		3	2	0.5	1	4976	9952	9931	19862	0.1	218472	9.09	19639	0.0183
				0.5	2	4955	9910			0.1				
	Blank (boiled microsomes)	1	2	0.5	1	57	114	120	240	0.1	218472	0.11		0
				0.5	2	63	126			0.1				
		2	2	0.5	1	54	108	106	212	0.1	218472	0.10		0
				0.5	2	52	104			0.1				
		3	2	0.5	1	54	108	109	218	0.1	218472	0.10		0
				0.5	2	55	110			0.1				
	No-NADP	1	2	0.5	1	16431	32862	32966	65932	0.1	218472	30.18	65709	0.0614
				0.5	2	16535	33070			0.1				
		2	2	0.5	1	16372	32744	32881	65762	0.1	218472	30.10	65539	0.0612
				0.5	2	16509	33018			0.1				
		3	2	0.5	1	16590	33180	33802	67604	0.1	218472	30.94	67381	0.0629
				0.5	2									

Table 1B. Replicate 1: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (μL)	Final volume of diluted microsomes (μL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.05	Complete	1	47	64	30000	0.100	1	0.050	15	0.0511	0.0051
		2	47	64	30000	0.100	1	0.050	15	0.0527	0.0053
	Inhibition	1	47	64	30000	0.100	1	0.050	15	0.0123	0.0012
		2	47	64	30000	0.100	1	0.050	15	0.0128	0.0013
		3	47	64	30000	0.100	1	0.050	15	0.0122	0.0012
	Blank (boiled microsomes)	1	47	64	30000	0.100	1	0.050	15	0.0000	0.0000
		2	47	64	30000	0.100	1	0.050	15	0.0000	0.0000
		3	47	64	30000	0.100	1	0.050	15	0.0000	0.0000
	No-NADP	1	47	64	30000	0.100	1	0.050	15	0.0408	0.0041
		2	47	64	30000	0.100	1	0.050	15	0.0407	0.0041
		3	47	64	30000	0.100	1	0.050	15	0.0418	0.0042

Table 2A. Replicate I: Calculation of Aromatase Activity (0.025 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.025	Complete	1	2	0.5	1	9856	19712	19706	39412	0.1	218472	18.04	39162	0.036572
				0.5	2	9850	19700							
		2	2	0.5	1	10070	20140	19968	39936	0.1	218472	18.28	39686	0.037061
				0.5	2	9898	19796							
		3	2	0.5	1	10427	20854	20711	41422	0.1	218472	18.96	41172	0.038449
					2	10284	20568							
	Inhibition	1	2	0.5	1	2510	5020	5007	10014	0.1	218472	4.58	9764	0.009118
				0.5	2	2497	4994							
		2	2	0.5	1	2386	4772	4737	9474	0.1	218472	4.34	9224	0.008614
					2	2351	4702							
		3	2	0.5	1	2413	4826	4886	9772	0.1	218472	4.47	9522	0.008892
					2	2473	4946							
	Blank	1	2	0.5	1	61	122	126	252	0.1	218472	0.12		0
				0.5	2	65	130							
		2	2	0.5	1	63	126	132	264	0.1	218472	0.12		0
					2	69	138							
		3	2	0.5	1	55	110	117	234	0.1	218472	0.11		0
					2	62	124							
	No-NADP	1	2	0.5	1	7935	15870	15925	31850	0.1	218472	14.58	31600	0.02951
				0.5	2	7990	15980							
		2	2	0.5	1	7537	15074	15104	30208	0.1	218472	13.83	29958	0.027977
					2	7567	15134							
		3	2	0.5	1	7888	15776	15586	31172	0.1	218472	14.27	30922	0.028877
					2	7698	15396							

Table 2B. Replicate I: Calculation of Aromatase Activity (0.025 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (μL)	Final volume of diluted microsomes (μL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.025	Complete	1	47	64	60000	0.050	1	0.025	15	0.0486	0.0024
		2	47	64	60000	0.050	1	0.025	15	0.0493	0.0025
		3	47	64	60000	0.050	1	0.025	15	0.0511	0.0026
	Inhibition	1	47	64	60000	0.050	1	0.025	15	0.0121	0.0006
		2	47	64	60000	0.050	1	0.025	15	0.0115	0.0006
		3	47	64	60000	0.050	1	0.025	15	0.0118	0.0006
	Blank	1	47	64	60000	0.050	1	0.025	15	0.0000	0.0000
		2	47	64	60000	0.050	1	0.025	15	0.0000	0.0000
		3	47	64	60000	0.050	1	0.025	15	0.0000	0.0000
No-NADP	1	47	64	60000	0.050	1	0.025	15	0.0392	0.0020	
	2	47	64	60000	0.050	1	0.025	15	0.0372	0.0019	
	3	47	64	60000	0.050	1	0.025	15	0.0384	0.0019	

Table 3A. Replicate I: Calculation of Aromatase Activity (0.0125 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.0125	Complete	1	2	0.5	1	4980	9960	10011	20022	0.1	218472	9.16	19723	0.018418
				0.5	2	5031	10062			0.1				
		2	2	0.5	1	4943	9886	9885	19770	0.1	218472	9.05	19471	0.018183
				0.5	2	4942	9884			0.1				
		3	2	0.5	1	4882	9764	10003	20006	0.1	218472	9.16	19707	0.018404
				0.5	2	5121	10242			0.1				
Inhibition	1	2	0.5	1	1169	2338	2385	4770	0.1	218472	2.18	4471	0.004175	
				2	1216	2432			0.1					
		2	2	0.5	1	1183	2366	2365	4730	0.1	218472	2.17	4431	0.004138
				0.5	2	1182	2364			0.1				
		3	2	0.5	1	1210	2420	2417	4834	0.1	218472	2.21	4535	0.004235
				0.5	2	1207	2414			0.1				
Blank	1	2	0.5	1	74	148	148	296	0.1	218472	0.14		0	
				2	74	148			0.1					
		2	2	0.5	1	82	164	151	302	0.1	218472	0.14		0
				0.5	2	69	138			0.1				
		3	2	0.5	1					0.1	218472			
				0.5	2					0.1				
No-NADP	1	2	0.5	1	4007	8014	8047	16094	0.1	218472	7.37	15795	0.01475	
				2	4040	8080			0.1					
		2	2	0.5	1	4023	8046	8003	16006	0.1	218472	7.33	15707	0.014668
				0.5	2	3980	7960			0.1				
		3	2	0.5	1	3732	7464	7468	14936	0.1	218472	6.84	14637	0.013669
				0.5	2	3736	7472			0.1				

Table 3B. Replicate I: Calculation of Aromatase Activity (0.0125 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (μL)	Final volume of diluted microsomes (μL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.0125	Complete	1	47	64	120000	0.025	1	0.013	15	0.0490	0.0012
		2	47	64	120000	0.025	1	0.013	15	0.0484	0.0012
		3	47	64	120000	0.025	1	0.013	15	0.0489	0.0012
	Inhibition	1	47	64	120000	0.025	1	0.013	15	0.0111	0.0003
		2	47	64	120000	0.025	1	0.013	15	0.0110	0.0003
		3	47	64	120000	0.025	1	0.013	15	0.0113	0.0003
	Blank	1	47	64	120000	0.025	1	0.013	15	0.0000	0.0000
		2	47	64	120000	0.025	1	0.013	15	0.0000	0.0000
		3	47	64	120000	0.025	1	0.013	15		
No-NADP	1	47	64	120000	0.025	1	0.013	15	0.0392	0.0010	
	2	47	64	120000	0.025	1	0.013	15	0.0390	0.0010	
	3	47	64	120000	0.025	1	0.013	15	0.0364	0.0009	

Table 4. Summary of Aromatase Activity

[protein] mg/mL	Product formation rate (nmol/min)		Aromatase activity (nmol/mg/min)				% Inhibition
	Complete	Inhibited	Complete		Inhibited		
			Activity	SD	Activity	SD	
0.05	0.0052	0.0012	0.0518	0.0008	0.0124	0.0003	76.0
0.025	0.0025	0.0006	0.0497	0.0013	0.0118	0.0003	76.2
0.0125	0.0012	0.0003	0.0488	0.0004	0.0111	0.0001	77.2

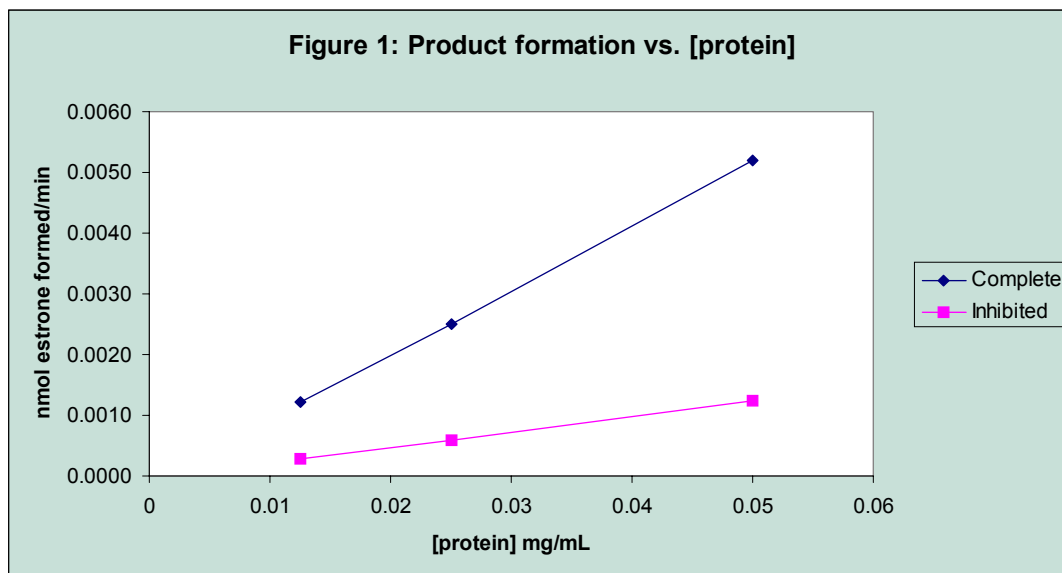


Table 5A. Follow-on Experiment: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.05	Complete	1	2	0.5	1	22700	45400	45099	90198	0.1	199816	45.14	89921	0.090733
				0.5	2	22399	44798			0.1				
		2	2	0.5	1	22747	45494	44590	89180	0.1	199816	44.63	88903	0.089706
	0.5			2	21843	43686			0.1					
	3		2	0.5	1	22700	45400	45330	90660	0.1	199816	45.37	90383	0.0912
		0.5		2	22630	45260			0.1					
-NADP		1	2	0.5	1	677	1354	1320	2640	0.1	199816	1.32	2363	0.002385
	0.5			2	643	1286			0.1					
	2		2	0.5	1	841	1682	1679	3358	0.1	199816	1.68	3081	0.003109
		0.5		2	838	1676			0.1					
		3	2	0.5	1	2157	4314	4230	8460	0.1	199816	4.23	8183	0.008257
	0.5			2	2073	4146			0.1					
-Regen system	1		2	0.5	1	54	108	127	254	0.1	199816	0.13		0
		0.5		2	73	146			0.1					
		2	2	0.5	1	76	152	141	282	0.1	199816	0.14		0
	0.5			2	65	130			0.1					
	3		2	0.5	1	69	138	147	294	0.1	199816	0.15		0
		0.5		2	78	156			0.1					

Table 5B. Follow-on Experiment: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.05	Complete	1	47	64	30000	0.100	1	0.050	15	0.0603	0.0060
		2	47	64	30000	0.100	1	0.050	15	0.0596	0.0060
		3	47	64	30000	0.100	1	0.050	15	0.0606	0.0061
	-NADP	1	47	64	30000	0.100	1	0.050	15	0.0016	0.0002
		2	47	64	30000	0.100	1	0.050	15	0.0021	0.0002
		3	47	64	30000	0.100	1	0.050	15	0.0055	0.0006
	-Regen system	1	47	64	30000	0.100	1	0.050	15	0.0000	0.0000
		2	47	64	30000	0.100	1	0.050	15	0.0000	0.0000
		3	47	64	30000	0.100	1	0.050	15	0.0000	0.0000

Table 6A. Follow-on Experiment: Calculation of Aromatase Activity (0.025 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.05	Complete	1	2	0.5	1	10957	21914	21926	43852	0.1	199816	21.95	43604	0.043998
				0.5	2	10969	21938			0.1				
		2	2	0.5	1	10953	21906	21496	42992	0.1	199816	21.52	42744	0.04313
				0.5	2	10543	21086			0.1				
		3	2	0.5	1	10531	21062	21007	42014	0.1	199816	21.03	41766	0.042143
				0.5	2	10476	20952			0.1				
	-NADP	1	2	0.5	1	3021	6042	5956	11912	0.1	199816	5.96	11664	0.011769
				0.5	2	2935	5870			0.1				
		2	2	0.5	1	1822	3644	3632	7264	0.1	199816	3.64	7016	0.007079
				0.5	2	1810	3620			0.1				
		3	2	0.5	1	2458	4916	4939	9878	0.1	199816	4.94	9630	0.009717
				0.5	2	2481	4962			0.1				
-Regen system	1	2	0.5	1	72	144	128	256	0.1	199816	0.13		0	
			0.5	2	56	112			0.1					
	2	2	0.5	1	55	110	120	240	0.1	199816	0.12		0	
			0.5	2	65	130			0.1					
	3	2	0.5	1	56	112	124	248	0.1	199816	0.12		0	
			0.5	2	68	136			0.1					

Table 6B. Follow-on Experiment: Calculation of Aromatase Activity (0.025 mg/mL protein) (continued)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.05	Complete	1	47	64	60000	0.050	1	0.025	15	0.0585	0.0029
		2	47	64	60000	0.050	1	0.025	15	0.0574	0.0029
		3	47	64	60000	0.050	1	0.025	15	0.0560	0.0028
	-NADP	1	47	64	60000	0.050	1	0.025	15	0.0157	0.0008
		2	47	64	60000	0.050	1	0.025	15	0.0094	0.0005
		3	47	64	60000	0.050	1	0.025	15	0.0129	0.0006
	-Regen system	1	47	64	60000	0.050	1	0.025	15	0.0000	0.0000
		2	47	64	60000	0.050	1	0.025	15	0.0000	0.0000
		3	47	64	60000	0.050	1	0.025	15	0.0000	0.0000

Table 7A. Follow-on Experiment: Calculation of Aromatase Activity (0.0125 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.05	Complete	1	2	0.5	1	5199	10398	10512	21024	0.1	199816	10.52	20798	0.020986
				0.5	2	5313	10626			0.1				
		2	2	0.5	1	5124	10248	10291	20582	0.1	199816	10.30	20356	0.02054
				0.5	2	5167	10334			0.1				
		3	2	0.5	1	5028	10056	10030	20060	0.1	199816	10.04	19834	0.020013
				0.5	2	5002	10004			0.1				
-NADP		1	2	0.5	1	525	1050	1056	2112	0.1	199816	1.06	1886	0.001903
				0.5	2	531	1062			0.1				
		2	2	0.5	1	1177	2354	2378	4756	0.1	199816	2.38	4530	0.004571
				0.5	2	1201	2402			0.1				
		3	2	0.5	1	1313	2626	2560	5120	0.1	199816	2.56	4894	0.004938
				0.5	2	1247	2494			0.1				
-Regen system		1	2	0.5	1	63	126	127	254	0.1	199816	0.13		0
				0.5	2	64	128			0.1				
		2	2	0.5	1	59	118	112	224	0.1	199816	0.11		0
				0.5	2	53	106			0.1				
		3	2	0.5	1	51	102	100	200	0.1	199816	0.10		0
				0.5	2	49	98			0.1				

Table 7B. Follow-on Experiment: Calculation of Aromatase Activity (0.0125 mg/mL protein) continued

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.05	Complete	1	47	64	120000	0.025	1	0.013	15	0.0558	0.0014
		2	47	64	120000	0.025	1	0.013	15	0.0546	0.0014
		3	47	64	120000	0.025	1	0.013	15	0.0532	0.0013
	-NADP	1	47	64	120000	0.025	1	0.013	15	0.0051	0.0001
		2	47	64	120000	0.025	1	0.013	15	0.0122	0.0003
		3	47	64	120000	0.025	1	0.013	15	0.0131	0.0003
	-Regen system	1	47	64	120000	0.025	1	0.013	15	0.0000	0.0000
		2	47	64	120000	0.025	1	0.013	15	0.0000	0.0000
		3	47	64	120000	0.025	1	0.013	15	0.0000	0.0000

Table 8. Summary of Aromatase Activity for Follow-on Experiment

[protein] mg/mL	Product formation rate (nmol/min)	Aromatase activity (nmol/mg/min)	
		Mean	SD
0.05	0.0060	0.0602	0.0005
0.025	0.0029	0.0573	0.0012
0.0125	0.0014	0.0546	0.0013

Table 9A. Replicate 2: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.05	Complete	1	2	0.5	1	32654	65308	64723	129446	0.1	295336	43.83	129088	0.088319
				0.5	2	32069	64138			0.1				
		2	2	0.5	1	33859	67718	66896	133792	0.1	295336	45.30	133434	0.091293
				0.5	2	33037	66074			0.1				
		3	2	0.5	1	34261	68522	67070	134140	0.1	295336	45.42	133782	0.091531
				0.5	2	32809	65618			0.1				
	Inhibition	1	2	0.5	1	7436	14872	14856	29712	0.1	295336	10.06	29354	0.020083
				0.5	2	7420	14840			0.1				
		2	2	0.5	1	7581	15162	15411	30822	0.1	295336	10.44	30464	0.020843
				0.5	2	7830	15660			0.1				
		3	2	0.5	1	7740	15480	15364	30728	0.1	295336	10.40	30370	0.020779
				0.5	2	7624	15248			0.1				
Blank (boiled microsomes)	1	2	0.5	1	92	184	173	346	0.1	295336	0.12		0	
			0.5	2	81	162			0.1					
	2	2	0.5	1	85	170	162	324	0.1	295336	0.11		0	
			0.5	2	77	154			0.1					
	3	2	0.5	1	107	214	202	404	0.1	295336	0.14		0	
			0.5	2	95	190			0.1					
No-NADP	1	2	0.5	1	190	380	373	746	0.1	295336	0.25	388	0.000265	
			0.5	2	183	366			0.1					
	2	2	0.5	1	193	386	411	822	0.1	295336	0.28	464	0.000317	
			0.5	2	218	436			0.1					

(continued)

**Table 9A. Replicate 2: Calculation of Aromatase Activity (0.05 mg/mL protein)
(continued)**

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ³ H ₂ O formed
		3	2	0.5	1	3653	7306	7187	14374	0.1	295336	4.87	14016	0.009589
				0.5	2	3534	7068			0.1				
	- System	1	2	0.5	1	119	238	220	440	0.1	295336	0.15	82	5.61E-05
				0.5	2	101	202			0.1				
		2	2	0.5	1	101	202	198	396	0.1	295336	0.13	38	2.6E-05
				0.5	2	97	194			0.1				
		3	2	0.5	1	95	190	200	400	0.1	295336	0.14	42	2.87E-05
				0.5	2	105	210			0.1				
	+ NADPH	1	2	0.5	1	31704	63408	62923	125846	0.1	295336	42.61	125488	0.085856
				0.5	2	31219	62438			0.1				
		2	2	0.5	1	31807	63614	63269	126538	0.1	295336	42.85	126180	0.08633
				0.5	2	31462	62924			0.1				
		3	2	0.5	1	31520	63040	62261	124522	0.1	295336	42.16	124164	0.084951
				0.5	2	30741	61482			0.1				

Table 9B. Replicate 2: Calculation of Aromatase Activity (0.05 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.05	Complete	1	47	85	40000	0.100	1	0.050	15	0.0590	0.0059
		2	47	85	40000	0.100	1	0.050	15	0.0609	0.0061
		3	47	85	40000	0.100	1	0.050	15	0.0611	0.0061
	Inhibition	1	47	85	40000	0.100	1	0.050	15	0.0134	0.0013
		2	47	85	40000	0.100	1	0.050	15	0.0139	0.0014
		3	47	85	40000	0.100	1	0.050	15	0.0139	0.0014
	Blank (boiled microsomes)	1	47	85	40000	0.100	1	0.050	15	0.0000	0.0000
		2	47	85	40000	0.100	1	0.050	15	0.0000	0.0000
		3	47	85	40000	0.100	1	0.050	15	0.0000	0.0000
No-NADP	1	47	85	40000	0.100	1	0.050	15	0.0002	0.0000	
	2	47	85	40000	0.100	1	0.050	15	0.0002	0.0000	
	3	47	85	40000	0.100	1	0.050	15	0.0064	0.0006	
- System	1	47	85	40000	0.100	1	0.050	15	0.0000	0.0000	
	2	47	85	40000	0.100	1	0.050	15	0.0000	0.0000	
	3	47	85	40000	0.100	1	0.050	15	0.0000	0.0000	
+ NADPH	1	47	85	40000	0.100	1	0.050	15	0.0573	0.0057	
	2	47	85	40000	0.100	1	0.050	15	0.0576	0.0058	
	3	47	85	40000	0.100	1	0.050	15	0.0567	0.0057	

Table 10A. Replicate 2: Calculation of Aromatase Activity (0.025 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ³ H ₂ O formed
0.025	Complete	1	2	0.5	1	15317	30634	30471	60942	0.1	295336	20.63	60629	0.041481
				0.5	2	15154	30308			0.1				
		2	2	0.5	1	15451	30902	30970	61940	0.1	295336	20.97	61627	0.042164
	0.5			2	15519	31038			0.1					
	3	2	2	0.5	1	15675	31350	30779	61558	0.1	295336	20.84	61245	0.041903
				0.5	2	15104	30208			0.1				
Inhibition	1	2	0.5	1	1	3608	7216	7157	14314	0.1	295336	4.85	14001	0.009579
				2	2	3549	7098			0.1				
		2	2	0.5	1	3554	7108	7018	14036	0.1	295336	4.75	13723	0.009389
	0.5			2	3464	6928			0.1					
	3	2	2	0.5	1	3709	7418	7315	14630	0.1	295336	4.95	14317	0.009796
				0.5	2	3606	7212			0.1				
Blank	1	2	0.5	1	1	81	162	163	326	0.1	295336	0.11		0
				2	2	82	164			0.1				
		2	2	0.5	1	66	132	147	294	0.1	295336	0.10		0
	0.5			2	81	162			0.1					
	3	2	2	0.5	1	72	144	159	318	0.1	295336	0.11		0
				0.5	2	87	174			0.1				
No-NADP	1	2	0.5	1	1	216	432	447	894	0.1	295336	0.30	581	0.000398
				2	2	231	462			0.1				
	2	2	0.5	1	1	506	1012	1066	2132	0.1	295336	0.72	1819	0.001245
				2	2	560	1120			0.1				

(continued)

**Table 10A. Replicate 2: Calculation of Aromatase Activity (0.025 mg/mL protein)
(continued)**

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ³ H ₂ O formed
		3	2	0.5	1	197	394	393	786	0.1	295336	0.27	473	0.000324
				0.5	2	196	392			0.1				
	- System	1	2	0.5	1	89	178	186	372	0.1	295336	0.13	59	4.06E-05
				0.5	2	97	194			0.1				
		2	2	0.5	1	84	168	184	368	0.1	295336	0.12	55	3.79E-05
				0.5	2	100	200			0.1				
		3	2	0.5	1	121	242	229	458	0.1	295336	0.16	145	9.94E-05
				0.5	2	108	216			0.1				
	+ NADPH	1	2	0.5	1	14914	29828	29715	59430	0.1	295336	20.12	59117	0.040447
				0.5	2	14801	29602			0.1				
		2	2	0.5	1	14936	29872	29794	59588	0.1	295336	20.18	59275	0.040555
				0.5	2	14858	29716			0.1				
		3	2	0.5	1	14963	29926	29873	59746	0.1	295336	20.23	59433	0.040663
				0.5	2	14910	29820			0.1				

Table 10B. Replicate 2: Calculation of Aromatase Activity (0.025 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.025	Complete	1	47	85	80000	0.050	1	0.025	15	0.0554	0.0028
		2	47	85	80000	0.050	1	0.025	15	0.0563	0.0028
		3	47	85	80000	0.050	1	0.025	15	0.0559	0.0028
	Inhibition	1	47	85	80000	0.050	1	0.025	15	0.0128	0.0006
		2	47	85	80000	0.050	1	0.025	15	0.0125	0.0006
		3	47	85	80000	0.050	1	0.025	15	0.0131	0.0007
	Blank	1	47	85	80000	0.050	1	0.025	15	0.0000	0.0000
		2	47	85	80000	0.050	1	0.025	15	0.0000	0.0000
		3	47	85	80000	0.050	1	0.025	15	0.0000	0.0000
No-NADP	1	47	85	80000	0.050	1	0.025	15	0.0005	0.0000	
	2	47	85	80000	0.050	1	0.025	15	0.0017	0.0001	
	3	47	85	80000	0.050	1	0.025	15	0.0004	0.0000	
- System	1	47	85	80000	0.050	1	0.025	15	0.0001	0.0000	
	2	47	85	80000	0.050	1	0.025	15	0.0001	0.0000	
	3	47	85	80000	0.050	1	0.025	15	0.0001	0.0000	
+ NADPH	1	47	85	80000	0.050	1	0.025	15	0.0540	0.0027	
	2	47	85	80000	0.050	1	0.025	15	0.0541	0.0027	
	3	47	85	80000	0.050	1	0.025	15	0.0543	0.0027	

Table 11A. Replicate 2: Calculation of Aromatase Activity (0.0125 mg/mL protein)

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM in 2 mL reaction	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	Total nmol ³ H ₂ O formed in 2 mL reaction
0.0125	Complete	1	2	0.5	1	7473	14946	14674	29348	0.1	295336	9.94	29041	0.019869
				0.5	2	7201	14402			0.1				
		2	2	0.5	1	7576	15152	14928	29856	0.1	295336	10.11	29549	0.020217
				0.5	2	7352	14704			0.1				
		3	2	0.5	1	7515	15030	15044	30088	0.1	295336	10.19	29781	0.020375
				0.5	2	7529	15058			0.1				
	Inhibition	1	2	0.5	1	1851	3702	3696	7392	0.1	295336	2.50	7085	0.004847
				0.5	2	1845	3690			0.1				
		2	2	0.5	1	1889	3778	3712	7424	0.1	295336	2.51	7117	0.004869
				0.5	2	1823	3646			0.1				
		3	2	0.5	1	1741	3482	3381	6762	0.1	295336	2.29	6455	0.004416
				0.5	2	1640	3280			0.1				
Blank	1	2	0.5	1	84	168	156	312	0.1	295336	0.11		0	
			0.5	2	72	144			0.1					
	2	2	0.5	1	72	144	153	306	0.1	295336	0.10		0	
			0.5	2	81	162			0.1					
	3	2	0.5	1	69	138	152	304	0.1	295336	0.10		0	
			0.5	2	83	166			0.1					
No-NADP	1	2	0.5	1	760	1520	1521	3042	0.1	295336	1.03	2735	0.001871	
			0.5	2	761	1522			0.1					
	2	2	0.5	1	640	1280	1265	2530	0.1	295336	0.86	2223	0.001521	
			0.5	2	625	1250			0.1					

(continued)

**Table 11A. Replicate 2: Calculation of Aromatase Activity (0.0125 mg/mL protein)
(continued)**

Sample Info			Calculate DPM in aqueous portion after extraction							Calculate % turnover				
Sample ID (mg/mL)	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ³ H ₂ O formed
				0.5	2	1065	2130			0.1				
	- System	1	2	0.5	1	85	170	154	308	0.1	295336	0.10	1	4.56E-07
				0.5	2	69	138			0.1				
		2	2	0.5	1	99	198	183	366	0.1	295336	0.12	59	4.01E-05
				0.5	2	84	168			0.1				
		3	2	0.5	1	100	200	202	404	0.1	295336	0.14	97	6.61E-05
				0.5	2	102	204			0.1				
	+ NADPH	1	2	0.5	1	6987	13974	14207	28414	0.1	295336	9.62	28107	0.01923
				0.5	2	7220	14440			0.1				
		2	2	0.5	1	7258	14516	14519	29038	0.1	295336	9.83	28731	0.019657
				0.5	2	7261	14522			0.1				
		3	2	0.5	1	7028	14056	14076	28152	0.1	295336	9.53	27845	0.019051
				0.5	2	7048	14096			0.1				

Table 11B. Replicate 2: Calculation of Aromatase Activity (0.0125 mg/mL protein)

Sample Info			Calculate final assay protein concentration								
Sample ID (mg/mL)	Sample type	Replicate	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	Activity expressed independent of [protein] (nmol/min)
0.0125	Complete	1	47	85	160000	0.025	1	0.012	15	0.0531	0.0013
		2	47	85	160000	0.025	1	0.012	15	0.0540	0.0013
		3	47	85	160000	0.025	1	0.012	15	0.0544	0.0014
	Inhibition	1	47	85	160000	0.025	1	0.012	15	0.0129	0.0003
		2	47	85	160000	0.025	1	0.012	15	0.0130	0.0003
		3	47	85	160000	0.025	1	0.012	15	0.0118	0.0003
	Blank	1	47	85	160000	0.025	1	0.012	15	0.0000	0.0000
		2	47	85	160000	0.025	1	0.012	15	0.0000	0.0000
		3	47	85	160000	0.025	1	0.012	15	0.0000	0.0000
	No-NADP	1	47	85	160000	0.025	1	0.012	15	0.0050	0.0001
		2	47	85	160000	0.025	1	0.012	15	0.0041	0.0001
		3	47	85	160000	0.025	1	0.012	15	0.0075	0.0002
	- System	1	47	85	160000	0.025	1	0.012	15	0.0000	0.0000
		2	47	85	160000	0.025	1	0.012	15	0.0001	0.0000
		3	47	85	160000	0.025	1	0.012	15	0.0002	0.0000
	+ NADPH	1	47	85	160000	0.025	1	0.012	15	0.0513	0.0013
		2	47	85	160000	0.025	1	0.012	15	0.0525	0.0013
		3	47	85	160000	0.025	1	0.012	15	0.0509	0.0013

Table 12. Summary of Aromatase Activity

[protein] mg/mL	Product formation rate (nmol/min)		Aromatase activity (nmol/mg/min)				% Inhibition
	Complete	Inhibited	Complete		Inhibited		
			Activity	SD	Activity	SD	
0.05	0.0060	0.0014	0.0603	0.0012	0.0137	0.0003	77.2
0.025	0.0028	0.0006	0.0559	0.0005	0.0128	0.0003	77.1
0.0125	0.0013	0.0003	0.0538	0.0007	0.0126	0.0007	76.6

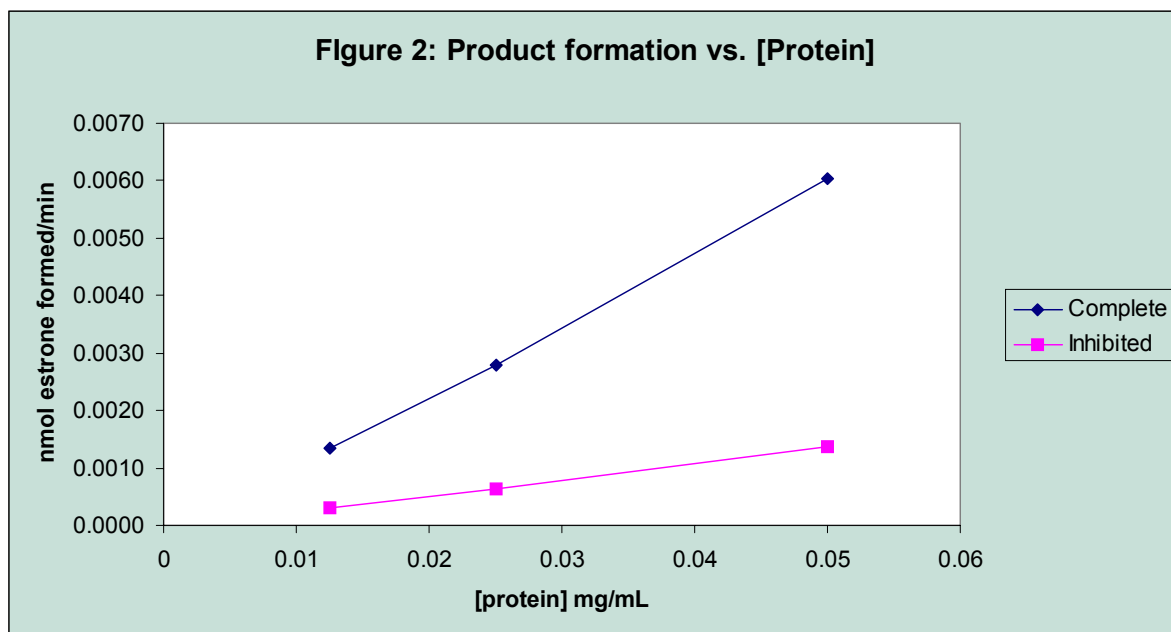


Table 13. Product Formation Rates, Substrate and Inhibition Percentages

Protein Concentration (mg/mL)	Experiment ID	Product formation rate (nmol/min)	% conversion of substrate to product	% inhibition
Complete system				
0.05	Replicate 1	0.0052	38.29	NA
	Follow-on	0.0060	45.05	
	Replicate 2	0.0060	44.85	
	Mean (SD)	0.0058 (0.0004)	42.73 (3.38)	
0.025	Replicate 1	0.0025	18.43	
	Follow-on	0.0029	21.50	
	Replicate 2	0.0028	20.82	
	Mean (SD)	0.0027 (0.0002)	20.25 (1.44)	
0.0125	Replicate 1	0.0012	9.12	
	Follow-on	0.0014	10.29	
	Replicate 2	0.0013	10.08	
	Mean (SD)	0.0013 (0.0001)	9.83 (0.56)	
Inhibited system				
0.05	Replicate 1	0.0012	9.25	76.0
	Replicate 2	0.0014	10.30	77.2
	Mean (SD)	0.0013 (0.0001)	9.78 (0.61)	
0.025	Replicate 1	0.0006	4.46	76.2
	Replicate 2	0.0006	4.85	77.1
	Mean (SD)	0.0006 (0.0000)	4.66 (0.23)	
0.0125	Replicate 1	0.0003	2.19	77.2
	Replicate 2	0.0003	2.43	76.6
	Mean (SD)	0.0003 (0.0000)	2.33 (0.16)	

Table 14. Average Aromatase Activity (nmol/mg/min)

Conditions	Replicate 1	Follow-on	Replicate 2	Mean (SD)
0.05 mg/mL protein				
Complete	0.0518	0.0602	0.0603	0.0574 (0.0043)
Inhibited	0.0124		0.0137	0.0131 (0.0008)
- NADP	0.0411	0.0030	0.0023	0.0155 (0.0193)
- System			0.0000	0.0000 (0.0000)
+ NADPH			0.0572	0.0572 (0.0005)
0.025 mg/mL protein				
Complete	0.0497	0.0573	0.0559	0.0543 (0.0036)
Inhibited	0.0118		0.0128	0.0123 (0.0006)
- NADP	0.0383	0.0127	0.0009	0.0173 (0.0166)
- System			0.0001	0.0001 (0.0000)
+ NADPH			0.0541	0.0541 (0.0001)
0.0125 mg/mL protein				
Complete	0.0488	0.0546	0.0538	0.0524 (0.0028)
Inhibited	0.0111		0.0126	0.0119 (0.0009)
- NADP	0.0382	0.0101	0.0055	0.0179 (0.0155)
- System			0.0001	0.0001 (0.0001)
+ NADPH			0.0516	0.0516 (0.0008)