TECHNICAL MANUAL

CYP19A (Aromatase) Activity Assay

- **SKU CODE:** ARMA00165
- **SIZE:** 200 Reactions
- **DETECTION PRINCIPLE:** Fluorometric
- Research-Use-Only

Revised: 06/2026

1. Introduction

CYP19A is responsible for the biosynthesis of estrogens from androgens. It is expressed in multiple tissues, including gonads, placenta, brain, adipose tissue and bone, allowing for localized estrogen production. Aromatase activity is tightly regulated by tissue-specific promoters, controlled by various hormones and cytokines. This complex regulation allows for fine-tuned estrogen production in different tissues. Instances of CYP19A influence on metabolism are in breast cancer, osteoporosis, gynecomastia and hypogonadism, and in obesity and metabolic syndrome. Inhibitors of CYP19A are finding increased use for a variety of medical conditions. Assay Genie's Aromatase Activity Assay is a simple, sensitive way to quantitate enzyme activity in a variety of biological sample types, with a sensitivity in the low nano-Unit range.

2. Assay Principle

In the presence of CYP19A and NADPH, a select substrate is dealkylated to a fluorescent product. The rate of increase in fluorescence is directly proportional to CYP19A activity.

3. Kit Components

Component	Quantity	Colour	Code
Assay Buffer	100 ml	NM	ARMA00165A
Fluorescein Standard	500 µl	Yellow	ARMA00165B
Aromatase Inhibitor	Lyophilised	Blue	ARMA00165C
NADPH Regenerating System	Lyophilised	Green	ARMA00165D
NADP	Lyophilised	Amber	ARMA00165E
Dibenzylfluorescein	Lyophilised	Red	ARMA00165F
Active Aromatase	Lyophilised	Violet	ARMA00165G

4. Storage and Handling

Store unopened kit at -20°C. Centrifuge all vials for a few seconds before opening. Bring all assay components to room temperature before using.

Assay Buffer: Ready to use as supplied. Store at 4°C.

Fluorescein Standard: Comes as a 100 nM solution. Store at 4°C.

Aromatase Inhibitor: Dissolve in 55 µl of acetonitrile, giving a 1 mM stock solution. Store at -20°C. Transfer 10 µl of the 1 mM stock solution to 990 µl of Assay Buffer to make a 10 µM solution for use as an inhibitor solution.

NADPH Regenerating System: Dissolve in 440 µl Assay Buffer, divide into convenient portions and store at -20°C to avoid repeated freeze/thaw cycles.

NADP: Dissolve in 220 µl Assay Buffer. Store at -20°C.

Dibenzylfluorescein: Dissolve in 55 µl acetonitrile giving a 1 mM solution. Store at -20°C.

Active Aromatase: Reconstitute only immediately before use, with 230 µl Assay Buffer. Add 20 µl of NADPH Regenerating System and mix thoroughly. Divide into convenient portions and store at -80°C to avoid repeated freeze/thaw cycles. Aromatase loses ~10% activity per week at -80°C. Thaw a portion rapidly at 37°C, keep on ice and use the same day.

5. Assay Protocol

1. Turn on a plate reader and set to 37°C. Collect data every 30-60 seconds for 30-60 minutes using 490 nm excitation, 535 nm emission.
2. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the 100 nM Standard into a series of wells in a 96-well plate, giving 0, 0.5, 1, 1.5, 2 and 2.5 pmoles of standard, respectively. Adjust the wells to 100 µl with Assay Buffer.
3. Sample Preparation: Microsomal preparations may be purchased commercially or prepared from tissue or cultured cells using established procedures. Alternatively, an enriched lysate can be prepared. Homogenize 50 mg tissue or 5 × 10^6 cells in 500 µl ice-cold Assay Buffer, on ice. Centrifuge at 16,000 × g for 10 minutes at 4°C. Transfer the supernatant to a fresh tube and place on ice. Homogenate supernatant can be stored at -80°C, adding a protease inhibitor before storage.

The effect of proposed inhibitors can be evaluated after dissolution in an appropriate solvent at 100X the final test concentration. Dilute to 5X the final test concentration with Assay Buffer. Run each sample in duplicate with the 2nd well used as a solvent control well.

Note: DMSO can inhibit aromatase at concentrations ≥ 0.25%. Acetonitrile shows much less inhibitory activity at or below 1%.

4. Preincubation: Each well will require 70 µl of different preincubation mixtures. Besides the Sample Mix, other reagent mixtures are needed to provide for various control wells, including Background Control, Inhibitor Control and Positive Control wells. Prepare sufficient materials for the number of each sample and control type to be run.

For each sample and control well, prepare a 2X aromatase Reaction Mix: add 2 µl of NADPH Regenerating System to each (2-48 µl) sample, for use in one well. Prepare each sample type in bulk (10 wells of a 25 µl sample would be 250 µl of sample plus 20 µl of NADPH Regenerating solution, mixed, then 27 µl transferred to each well and the well volumes adjusted to 50 µl with Assay Buffer). The amount of sample required

will vary based upon the activity of the sample. An initial estimate of protein needed per well is 25-50 µg of microsomal protein, 50-200 µg for S9 or other lysates.

In addition to test samples, Background and Inhibitor Controls should be included. A Positive Control and PC + Inhibitor well will show the true behavior of CYP19A under the experimental conditions. Adjust all wells to 70 µl with Assay Buffer. For measurement of prospective inhibitors, substitute Assay Buffer in the table below with a 5X solution of inhibitor, prepared in step 3 above.

Reagent	Test Sample	Inhib Control	Bkgd Control	Pos. Control	PC + Inhib
2X Reaction Mix (step 4)	50 µl	50 µl	—	—	—
Active Aromatase	—	—	—	25 µl	25 µl
Aromatase Inhibitor (10 µM)	—	10 µl	—	—	10 µl
Inhibitor Solvent	—	—	—	—	—
Assay Buffer (or Test Inhibitor)	20 µl	10 µl	70 µl	45 µl	35 µl

Add the various mixes to the appropriate wells and preincubate the plate for 15 minutes at 37°C to allow for inhibitor binding to occur.

5. Initiate Reaction: While the preincubation is occurring, prepare the substrate/NADP reaction which will start the reaction. For each 10 wells to be run, combine:

   Assay Buffer: 288.8 µl

   Dibenzylfluorescein: 1.2 µl

   NADP solution: 10.0 µl

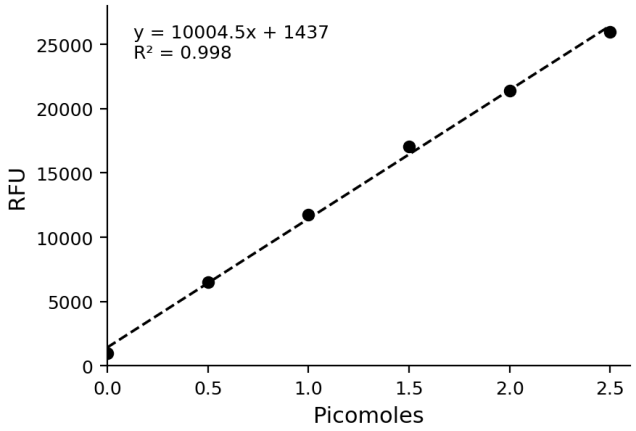
   Total: 300.0 µl

   Scale this recipe as needed for the number of wells to be run. Add 30 µl per well using a multiple channel pipette to initiate the wells as simultaneously as possible.

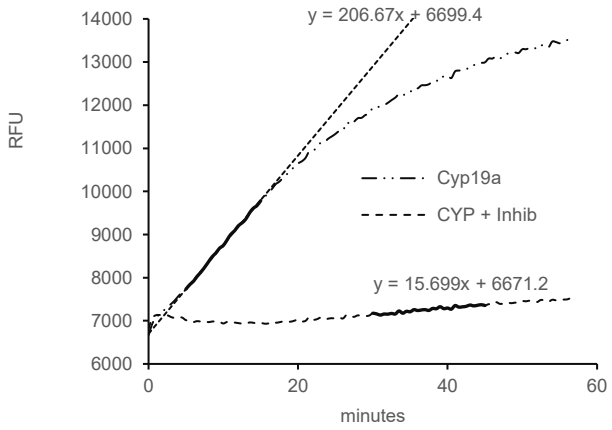
   - Active Aromatase particles are slightly dense and can settle - mix before transferring to wells.
   - The substrate is not specific to CYP19A but is also metabolized by 2C8, 2C9 and 2C19, hence the addition of the CYP19A inhibitor to be able to correct for the other activities in samples. The inhibitor concentration has been chosen to be more than 100-fold greater for CYP19A than for the other CYPs present.
   - Warming the Assay Buffer to 37°C will minimize fluorescence changes due to thermal equilibration.
6. Measurement: Immediately place the plate in the plate reader and begin measuring the fluorescence as set up in step 1 and collect fluorescence data for 30-60 minutes.

6. Typical Results

Standard (pmole)	Raw RFU	Background-Corrected RFU
0	984	0
0.5	6487	5502
1.0	11731	10747
1.5	17063	16079
2.0	21417	20433
2.5	25975	24991



Standard curve (representative data).



Representative kinetic traces (RFU vs. time).

7. Calculations

Subtract the 0 standard from all other standard readings. Plot the standard curve and determine the slope of the standard curve. For all background control, enzyme and inhibitor control wells, identify a linear portion of the data curve. Determine the slope of the different curves. To determine enzyme activity in the well, subtract the slope of the background control well from the slope of the enzyme well. Take the final datapoint

for all background, enzyme and inhibitor curves and determine the ratio of RFU after NAOH addition / RFU before NAOH.

For inhibition data, subtract the slope of the background control well from the slope of the inhibitor control well.

Divide the background-corrected values by the slope of the standard curve to convert RFU/minute to picomoles/minute of product formation in the well.

For inhibition studies: % inhibition = [1 − (background-corrected inhibitor slope) / (background-corrected enzyme slope)] × 100

To convert the enzyme activity data in the well back to activity in the original samples:

A. Divide the adjusted background-corrected enzyme rate by the volume of sample applied to the well = picomoles/minute per µl of sample.

B. Multiply by the total amount of supernatant obtained in step 3 above = total picomoles/minute (µUnits) of enzyme in the sample.

C. Divide by the mass of tissue or number of cells used to prepare the sample = µUnits of enzyme activity/mg sample (or per number of cells).

8. Statement

This kit is for Research Use Only. Not to be used for diagnostic or therapeutic procedures. Please read the instructions carefully and follow them strictly during the experiments. Protective measures should be taken, including wearing a lab coat and gloves.

Notes

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.