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ab273306 Aromatase (CYP19A) Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273306
(use https://www.abcam.cn/ab273306 for china, or
https://www.abcam.co.jp/ab273306 for Japan)

For the determination of Aromatase activity in human and animal tissue microsomes, cell and tissue lysates and heterologously expressed recombinant aromatase preparations.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Aromatase Activity Assay Kit (Fluorometric) (ab273306) enables rapid measurement of native or recombinant aromatase activity in biological samples such as placental microsomes.

The assay utilizes a fluorogenic substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 488/527 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence.

A highly selective aromatase inhibitor is provided for determination of aromatase activity in heterogeneous biological samples, where other CYP isozymes may contribute to substrate metabolism. The inhibitor displays greater than 100-fold selectivity for aromatase over other enzymes, ensuring targeted inhibition. Aromatase specific activity is calculated by running parallel reactions in the presence and absence of the inhibitor and subtracting any residual activity detected with the inhibitor present.

The kit contains a complete set of reagents sufficient for 50 sets of paired reactions (in the presence and absence of inhibitor) and can detect a minimum of 25 nU aromatase activity.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Prepare Reaction Mixes and add to the appropriate wells.

Incubate the plate for at least 10 mins at 37°C to allow the inhibitor letrozole or any test ligands to interact with aromatase.

Prepare Aromatase Substrate/NADP+ mixture and add to each well.

Immediately measure fluorescence in kinetic mode for 60 mins at 37ºC.

Determine Aromatase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Aromatase Assay Buffer	100 mL	-20°C
Aromatase Fluorescence Standard	50 µL	-20°C
Aromatase Inhibitor	1 vial	-20°C
100X NADPH Generating System II	1 vial	-20°C
Beta-NADP Stock	1 vial	-20°C
Aromatase Substrate	1 vial	-20°C
Recombinant Human Aromatase	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) acetonitrile and DMSO
- White 96-well plates with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

9.1 **Aromatase Assay Buffer:**
Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

9.2 **Aromatase Substrate:**
Briefly spin the vial. Reconstitute with 55 µl anhydrous reagent-grade acetonitrile and vortex until fully dissolved to obtain a 1 mM stock solution. Store at -20°C. Allow the vial to warm to room temperature before opening and promptly retighten cap after use to avoid absorption of airborne moisture.

9.3 **Aromatase inhibitor:**
Briefly spin the vial. Reconstitute in 55 µl of acetonitrile and vortex until fully dissolved to yield a 1 mM stock solution. To obtain a 5 µM working solution of letrozole (5X final concentration), add 5 µl of the 1 mM stock solution to 995 µl of Aromatase Assay Buffer. The 5 µM working solution should be stored at -20°C and is stable for 2 freeze/thaw cycles. The stock solution is stable for 2 months at -20°C.

9.4 **Aromatase Fluorescence Standard:**
Provided as a 1 mM stock solution in DMSO. Prior to use, warm to room temperature, vortex until dissolved and briefly spin vial. Store at -20°C, stable for at least 3 freeze/thaw cycles.

9.5 **Beta-NADP Stock :**
Briefly spin the vial. Dissolve in 110 µl Aromatase Assay Buffer and vortex thoroughly to yield a 100X stock solution of NADP+. Store at -20°C, stable for at least 3 freeze/thaw cycles.

9.6 **Recombinant Human Aromatase:**
Do not reconstitute until ready to use. Briefly spin the vial. Reconstitute with 230 µl Aromatase Assay Buffer and add 20 µl of 100X NADPH Generating System II. Mix thoroughly to ensure a homogenous solution (the solution will have a slightly opaque, milky appearance), aliquot and store at -80°C. Avoid repeated freeze/thaw cycles and use aliquots within one month (the Recombinant Human Aromatase will lose approximately 10% activity per week when stored at -80°C).

Thaw aliquots rapidly at 37°C and place on ice until use (thawed aliquots should be used within 4 hours).

9.7 **100X NADPH Generating System II:**
Briefly spin the vial. Reconstitute with 220 µl Aromatase Assay Buffer, aliquot and store at -20°C. Avoid repeated freeze/thaw cycles and keep on ice while in use.

10. Sample Preparation

– Standardized microsomal preparations may be purchased commercially (e.g. donor-pooled human placental microsomes) or prepared from tissue or cultured cells using a Microsome Isolation Kit.

Crude enriched lysates

10.1 A crude enriched lysate can be prepared: start with ~50 mg tissue or ~5 x 10^6 pelleted, pre-washed cells and homogenize in 500 µl ice-cold Aromatase Assay Buffer with a Dounce homogenizer on ice.

10.2 Incubate the homogenate on ice for 5 mins and then centrifuge at 15,000 x *g* for 15 mins in a refrigerated centrifuge at 4°C. Collect the resultant clarified supernatant for the assay in a new pre-chilled microfuge tube and store on ice until use (cell and tissue lysates can also be stored at -80°C in aliquots for future experiments). We recommend using the stored sample within one month.

Test Ligands

10.3 If desired, sample aromatase activity may be measured in the presence of test ligands. Test ligands should be dissolved into proper solvent to produce stock solutions (see note regarding solvent effects below). For each ligand, prepare a 5X solution by diluting in Aromatase Assay Buffer

Δ Note: If measuring aromatase activity in presence of ligands (other than the included Aromatase Inhibitor), run parallel solvent control well(s) to account for additional solvent in the reaction mix. Many commonly-used organic solvents can severely impact aromatase activity. Importantly, DMSO causes significant inhibition of aromatase at final concentrations ≥ 0.25% (v/v). Our assay is designed to use acetonitrile at a final concentration of ≤1% (v/v), which has been shown to have little impact on aromatase activity.

11. Standard Curve

11.1 Dilute the Aromatase Fluorescence Standard by adding 10 µl of the 1 mM stock to 990 µl Aromatase Assay Buffer to yield a 10 µM solution.

11.2 Mix 50 µl of the 10 µM solution with 950 µl Aromatase Assay Buffer to generate the final 0.5 pmole/µl (0.5 µM) Aromatase Fluorescence Standard.

11.3 Add 0, 2, 4, 6, 8, 12, 16 and 20 µl of the 0.5 pmole/µl Standard into a series of wells in a white 96-well plate, yielding 0, 1, 2, 3, 4, 6, 8 and 10 pmole/well Aromatase Fluorescence Standard.

11.4 Adjust the volume of each well to 100 µl with Aromatase Assay Buffer.

Standard #	0.5 µM Aromatase Fluorescence Standard (µL)	Aromatase Assay Buffer (µL)	Aromatase Fluorescence Standard (pmol/well)
1	0	100	0
2	2	98	1
3	4	96	2
4	6	94	3
5	8	92	4
6	12	88	6
7	16	84	8
8	20	80	10

11.5 Measure fluorescence at Ex/Em = 488/527 nm.

11.6 Subtract the zero standard (0 pmole/well) reading from all of the standard readings,

11.7 Plot the background-subtracted values and calculate the slope of the standard curve.

12. Assay Procedure

– Since the reaction starts immediately after the addition of the Aromatase Substrate/NADP+ mix, it is essential to preconfigure the fluorescence microplate reader settings and use a multichannel pipette with a reagent reservoir to minimize lag time among wells.

Reaction Mix:

12.1 Prepare enough reagents for the number of reactions to be performed.

12.2 For each reaction, prepare a 2X concentrated aromatase reaction mix (as shown in table below) by combining 2-48 µl of sample and 2 µl of the 100X NADPH Generating System II in a 96-well plate and adjusting the final volume to 50 µl/reaction with Aromatase Assay Buffer.

Δ Note: The amount of sample per reaction and the dilution factor required will vary based upon the nature of the sample. For human placental microsomes, we recommend starting with 25-50 µg of microsomal protein per well.

Δ Note: For S9 fractions or other cellular lysates, the amount of protein required will be significantly higher. In this case, we recommend starting at 50-100 µg/well.

12.3 In addition to the test samples, prepare background control (no enzyme) and positive inhibition control (sample + 1 µM letrozole) wells.

12.4 If desired, you may also prepare positive control (PC) and PC + inhibitor wells using the Recombinant Human Aromatase and letrozole 5 µM solution (for a 1 µM final concentration).

12.5 Adjust the volume of test sample, positive inhibition control, background and positive control wells to 70 µl/well with Aromatase Assay Buffer.

12.6 For measurement of sample aromatase activity in the presence of test ligands, replace the Aromatase Assay Buffer with 5X concentrated solution of test ligand in Aromatase Assay Buffer.

	Test sample	Inhibitor Control	Background	Positive Control	Positive control + inhibitor
Aromatase Reaction Mix (2X)	50 µL	50 µL	---	---	---
Recombinant Human Aromatase	---	---	---	25 µL	25 µL
Aromatase Inhibitor	---	20 µL	---	---	20 µL
Aromatase Assay Buffer	20 µL	---	70 µL	45 µL	25 µL

12.7 Incubate the plate for 10 minutes at 37°C to allow the inhibitor letrozole or any test ligands to interact with aromatase. The preincubation time can be optimized for other test ligands depending on mechanism of action.

12.8 During the incubation, prepare an Aromatase Substrate/NADP+ mixture (3X) by adding 6 µl of the reconstituted 1 mM Aromatase Substrate stock solution and 50 µl of the reconstituted 10 mM β-NADP+ Stock (100X) to 1444 µl of Aromatase Assay Buffer for a total volume of 1.5 ml.

12.9 This preparation is sufficient for 50 reactions but can be scaled depending upon the number of reactions to be performed. Start the reaction by adding 30 µl of the Aromatase Substrate/NADP+ (3X) mixture to each well using a multichannel pipette, yielding a final reaction volume of 100µl/well.

Δ Note: The Recombinant Human Aromatase preparation may settle and should be thoroughly mixed before dispensing.

Δ Note: The Aromatase Substrate is also metabolized by CYP isoforms 2C8, 2C9 and 2C19, necessitating the use of the selective inhibitor letrozole to determine the contribution of aromatase in biological samples that express aromatase along with other CYPs.

The concentration of letrozole used for the positive inhibition control is >100-fold greater than the Ki for recombinant aromatase but is not high enough to affect other enzymatic targets.

Measurement

12.10 Immediately after the addition of the substrate, measure the fluorescence in kinetic mode at 37°C for 60 minutes (EX/EM = 488/527 nm)

12.11 While the assay can be performed in either endpoint or kinetic mode, we strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the content of active aromatase in the sample.

13. Calculations

- The Aromatase Substrate undergoes rapid photobleaching in aqueous solutions. This photophysical property may give rise to a sharp non-linear phase in first few minutes of the reaction progress curves (a lag phase). When calculating ΔF values, it is important to choose time points that occur after this initial lag phase, during the linear range of the reaction. In our experience, the linear phase begins roughly 5-10 mins after the initiation of the reaction.
- If the background control (BC) well rate calculation yields a negative value subtraction of the BC value may be ignored.

13.1 For each reaction well (including background and positive inhibition controls), choose two time points (T1 and T2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU1 and RFU2) and determine the change in fluorescence over the time interval: ΔF = RFU2 – RFU1.

13.2 Subtract the ΔF value of the background control (BC) from those of the test samples (S) and 1 µM letrozole positive inhibition control (I) to determine the background-corrected change in fluorescence intensity for each well.

13.3 If the positive inhibition control well rate calculation yields a negative value subtraction of the ΔFI may be ignored.

13.4 Calculate the specific fluorescence generated by aromatase activity by subtracting the positive inhibition control from each sample:

$$C_S = (\Delta F_S - \Delta F_{BC}) - (\Delta F_1 - \Delta F_{BC})$$

C_S = Fluorescence from Aromatase activity
ΔF_S = test sample fluorescence
ΔF_{BC} = Background control fluorescence
ΔF_1 = Positive inhibition

13.5 Aromatase metabolic activity is obtained by applying the CS values to the Aromatase Fluorescence Standard curve to get B pmole of substrate metabolized by aromatase during the reaction time.

$$Aromatase\ activity\ = \frac{B}{(\Delta t \text{ x } P)} = pmol/min/mg = \mu U/mg$$

B = Metabolite amount produced, calculated from the Standard Curve (pmol)
Δt = Reaction time ($T_2 - T_1$) (mins)
P = sample amount (mg)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

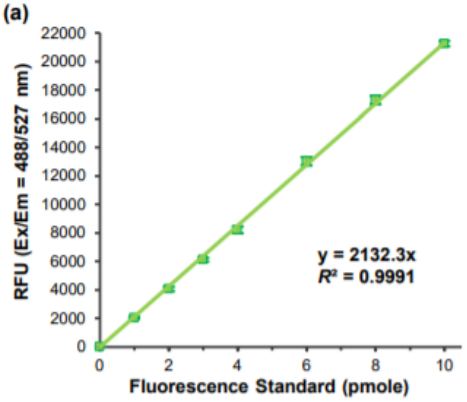

Figure 1.(a) Standard curve of Aromatase Substrate metabolite fluorescence. One mole of Aromatase Fluorescence Standard corresponds to the metabolism of one mole of Aromatase Substrate.

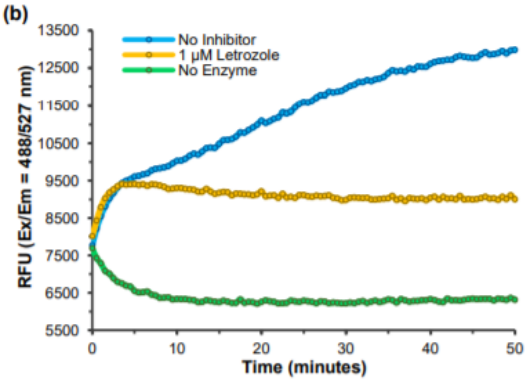

Figure 2. (b) Reaction kinetics of fluorogenic substrate metabolism in donor-pooled human placental microsomes (0.5 mg/mL) at 37°C in the presence and absence of the competitive aromatase inhibitor letrozole (the no inhibitor condition contained assay buffer with 0.2% acetonitrile as a solvent control)

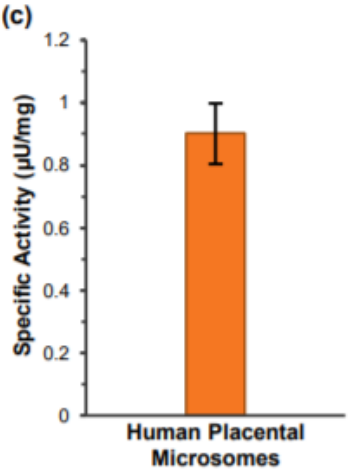

Figure 3. (c). Specific activity of aromatase in human placental microsome samples (mean ± SEM of four independent replicates). Assays were performed according to the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)