FABP4 Inhibitor/Ligand Screening Assay Kit

Item No. 10010231

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
10010377	Arachidonic Acid Control	1 vial/200 µl	-80°C
10010317	FABP Assay Buffer (10X)	1 vial/2 ml	-20°C
10010376	FABP Assay Detection Reagent (5X)	1 vial/1 ml	-20°C
10010318	FABP4 (human recombinant) Assay Reagent (5X)	1 vial/1 ml	-80°C
400017	96-Well Solid Plate (black)	2 plates	RT
400023	Foil Plate Covers	2 covers	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring fluorescence with excitation 370 ± 10 nm and emission 475 ± 10 nm.
2. Adjustable pipettes.
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable.
4. Microcentrifuge tubes (2 ml).

INTRODUCTION

Background

Fatty acid binding protein 4 (Adipocyte-FABP, Ap2, FABP4) is one of nine known cytosolic fatty acid binding proteins ranging in size from 14-15 kDa containing 127-132 amino acids.[1] Members of this protein family exhibit high affinity for small lipophilic ligands and were named according to the tissue from which they were initially isolated.[1] Studies suggest that FABPs are involved in the uptake and metabolism of fatty acids, in the maintenance of cellular membrane fatty acid levels, in intracellular trafficking of these substrates, in the modulation of specific enzymes of lipid metabolic pathways, and in the modulation of cell growth and differentiation.[2] FABP4 is highly expressed in adipocytes and is regulated by peroxisome-proliferator-activated receptor-γ (PPARγ) agonists, insulin and fatty acids. Recent studies using FABP4 gene deletion in mice indicate a dominant role for FABP4 in several chronic metabolic diseases. Therefore, inhibiting the function of FABP4 is a potential target for the treatment of metabolic diseases like diabetes and atherosclerosis.[3,4]

About This Assay

Cayman's FABP4 Inhibitor/Ligand Screening Assay Kit provides a simple, reproducible, and sensitive tool for the identification of FABP4 ligands. The Detection Reagent exhibits increased fluorescence when bound to FABP4. Binding of the Detection Reagent can be monitored by exciting at 370 nm and measuring the emission at 475 nm. Any strong ligand and/or inhibitor to FABP4 can displace the Detection Reagent thereby reducing the fluorescence. Arachidonic acid, a known ligand of FABP4, can displace the Detection Reagent with a IC_{50} value of 3 µM under the current assay conditions. For a more sensitive assay format please see the Appendix on page 17. Other ligands such as oleic acid, linolenic acid and docosahexaenoic acid can also displace the Detection Reagent by binding to FABP4.

PRE-ASSAY PREPARATION

Reagents Supplied

1. **Arachidonic Acid Control - (Item No. 10010377)**

   The vial contains 200 µl of 1.28 mM arachidonic acid in ethanol. Sufficient quantity is provided to prepare two control curves in duplicate. Store at -20°C.

2. **FABP Assay Buffer (10X) - (Item No. 10010317)**

   The vial contains 2 ml of 200 mM potassium phosphate, pH 7.2, containing 800 mM potassium chloride. One vial of Assay Buffer is sufficient for two 96-well plates. This solution should be thawed and stored at 4°C.

3. **FABP Assay Detection Reagent (5X) - (Item No. 10010376)**

   The vial contains 1 ml of Detection Reagent in ethanol. The stock solution is stable for at least six months when stored at -20°C. One vial is sufficient for two 96-well plates.

4. **FABP4 (human recombinant) Assay Reagent (5X) - (Item No. 10010318)**

   The vial contains 1 ml purified FABP4 in 50 mM sodium phosphate, pH 7.2, containing 100 mM sodium chloride and 20% glycerol. One vial is sufficient for two 96-well plates. Store at -20°C.

Sample Preparation

This assay has been tested for compatibility with various solvents which may be used for dissolving the inhibitor/ligand of interest. Organic solvents (up to 10% of the assay volume) such as ethanol, methanol or DMSO have shown to have no detrimental effects to the binding capability of ligands or the Detection Reagent. Dilution in one of these solvents is recommended.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

1. **FABP Assay Buffer (1X)**

   Dilute the FABP Assay Buffer (10X) by mixing 2 ml of 10X solution and 18 ml of HPLC-grade water.

2. **FABP Assay Detection Reagent (1X)**

   Prepare a working concentration of Detection Reagent by mixing 500 µl of Detection Reagent stock and 2 ml FABP Assay Buffer (1X). This is enough for one 96-well plate. *NOTE: The fluorescent compound is light sensitive, so avoid exposure to light sources. The solution is only stable for one day and should be kept on ice during use.*

3. **FABP4 Assay Protein (1X)**

   Prepare a working concentration of FABP4 protein by mixing 500 µl of FABP4 stock and 2 ml FABP Assay Buffer (1X). This is enough for one 96-well plate. *NOTE: The solution should be kept on ice during use.*

4. **Inhibitor/Ligand Preparation**

   Several different concentrations of inhibitor/ligand of interest should be prepared to test for binding. Compounds can be diluted in either ethanol, methanol or DMSO. Table 1, on page 9, should be used for making dilutions of the arachidonic acid controls.

Arachidonic Acid Preparation

For the determination of FABP4 binding to arachidonic acid, prepare controls as described below in Table 1. Label seven microcentrifuge tubes S1-S7 and add the indicated amount of ethanol to each tube. Serially dilute the arachidonic acid stock by adding the appropriate volume from the specified source.

Tube	Arachidonic Acid Concentration (µM)	Arachidonic Acid Source	Arachidonic Acid Volume (µl)	Ethanol (µl)	Arachidonic Acid Assay Concentration (µM)
S1	640	Stock	50	50	64
S2	160	Tube S1	25	75	16
S3	80	Tube S2	50	50	8
S4	40	Tube S3	50	50	4
S5	20	Tube S4	50	50	2
S6	10	Tube S5	50	50	1
S7	0	N/A	0	100	0

Table 1. Arachidonic Acid dilution instructions

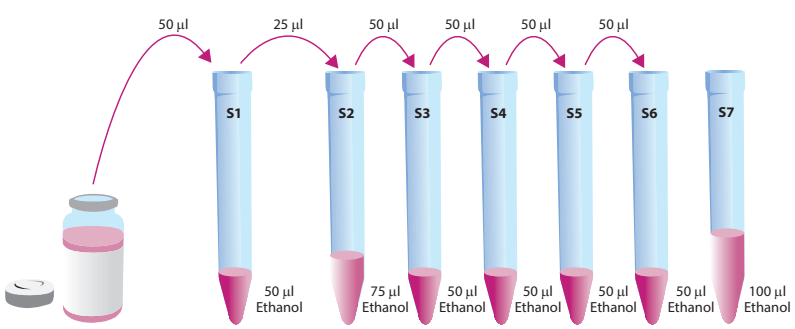


Figure 1. Preparation of Arachidonic Acid dilutions

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout for the arachidonic acid titration and samples is given below in Figure 2. It is suggested that all samples be assayed in duplicate. A template sheet is provided, on page 22, to record the contents of each well.

	⌐S¬		⌐A¬		⌐B¬		⌐C¬		⌐D¬		⌐E¬	
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk
B	S1	S1	A1	A1	B1	B1	C1	C1	D1	D1	E1	E1
C	S2	S2	A2	A2	B2	B2	C2	C2	D2	D2	E2	E2
D	S3	S3	A3	A3	B3	B3	C3	C3	D3	D3	E3	E3
E	S4	S4	A4	A4	B4	B4	C4	C4	D4	D4	E4	E4
F	S5	S5	A5	A5	B5	B5	C5	C5	D5	D5	E5	E5
G	S6	S6	A6	A6	B6	B6	C6	C6	D6	D6	E6	E6
H	S7	S7	A7	A7	B7	B7	C7	C7	D7	D7	E7	E7

Blk - Blank Wells
S1-S7 - Control Wells 1-7
A-E - Sample Wells 1-7

Figure 2. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the buffer, control, reagent, and protein.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The FABP Assay Buffer should be thawed and equilibrated to room temperature (21-24°C). All other reagents and samples should be kept on ice.
- The final volume of the assay is 100 µl in all wells.
- The incubation time is 15 minutes at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that all samples be assayed at least in duplicate or triplicate.
- Read the fluorescence emission at 475 nm following excitation at 370 nm using a plate reader.

Addition of Reagents

1. Add 40 µl of FABP Assay Buffer to each well.
2. Add 25 µl of FABP4 protein solution to each well. For blank wells, add 25 µl of FABP Assay Buffer instead.
3. Add 25 µl of Detection Reagent to each well.
4. Add 10 µl of each inhibitor/ligand dilution from tubes S1-S7 or A1-A7, etc. for test samples (see Table 1). For blank wells, add 10 µl of solvent used to dissolve inhibitor/ligand (ethanol, DMSO, etc.).
5. Cover the plate and incubate at room temperature for 10 minutes.
6. Read fluorescence at 475 nm after excitation at 370 nm using a plate reader. If these wavelengths are unavailable, a 10 nm shift is acceptable.

ANALYSIS

Calculations

1. Calculate the average fluorescence of each sample. Use this value for subsequent calculations.
2. For each sample set, subtract the blank value (well A) from each sample within that set (wells B through H). This is the background corrected fluorescence (BCF).
3. Divide the BCF of each sample by the maximum BCF (well H of that set) and multiply by 100%. This is the value in percent fluorescence units (% FU).
4. Plot the % FU values (from step 3 above) against the concentration of inhibitor/ligand used. An example is given in figure 3, on page 15.
5. To determine IC_{50} values, find the concentration of inhibitor/ligand that corresponds to 50% FU.

Performance Characteristics

Assay Range:

Under the standardized conditions of the assay, the dynamic range for arachidonic acid is 0-64 µM. However, this range can be adjusted for experimental samples based on affinity.

Representative Displacement Curve

The plot presented here is an example of the data seen using this assay; however, your results may vary.

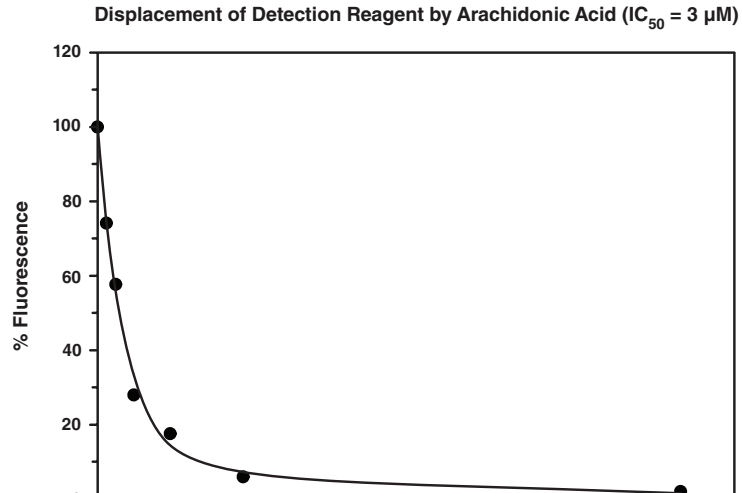


Figure 3. Typical displacement curve

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; titration does not match trend in example	A. Poor pipetting/technique B. Bubble in the well(s) C. Incomplete incubation period D. Inhibitor/ligand insoluble in buffer	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove the bubbles C. Reread plate after extended incubation D. Ensure sample dilutions were made in solvent or increase organic solvent volume in well
Titration showed no decrease in fluorescence	A. Concentrations of samples are outside dynamic range of assay B. Insoluble inhibitor/ligand	A. This compound is not a strong inhibitor/ligand; prepare fresh dilutions of sample at a higher concentration range B. Increase solvent percentage to dissolve the inhibitor/ligand
Fluorescence decreased too quickly to determine IC_{50}	Concentrations of samples are outside dynamic range of assay	This compound may be a strong inhibitor/ligand; prepare fresh dilutions of sample at a lower concentration range
Fluorescent values are not in detectable limit	A. Gain used was too high/low B. Fluorescent compound was exposed to too much light	A. Adjust gain B. Prepare fresh reagents C. Avoid exposing detection reagent to light

Appendix

Protocol for improving sensitivity of the FABP4 Inhibitor/Ligand Screening Assay Kit.

NOTE

This assay requires a highly sensitive fluorescence reader equipped to do excitation and emission wavelengths of 370 and 475 nm respectively. In the more sensitive assay format, slight variations in organic solvent can have a significant reduction in signal, therefore, we recommend that no more than 5-10% of organic solvent be used in this format.

Preparation of Assay-Specific Reagents for Modified Assay

1. **FABP Assay Buffer (1X)**

   Dilute the FABP Assay Buffer (10X) by mixing 2 ml of 10X solution and 18 ml of HPLC-grade water.

2. **FABP Assay Detection Reagent (1X)**

   Prepare a working concentration of Detection Reagent by mixing 100 µl of Detection Reagent (5X) and 2.4 ml FABP Assay Buffer (1X). This is enough for one 96-well plate. *NOTE: The fluorescent compound is light sensitive, so avoid exposure to light sources. The solution is only stable for one day and should be kept on ice during use.*

3. **FABP4 Assay Protein (1X)**

   Prepare a working concentration of FABP4 protein by mixing 125 µl of FABP4 (5X) and 2.375 ml FABP Assay Buffer (1X). This is enough for one 96-well plate. *NOTE: The solution should be kept on ice during use.*

4. **Inhibitor/ligand Preparation**

   Several different concentrations of inhibitor/ligand of interest should be prepared to test for binding. Compounds can be diluted in either ethanol, methanol, or DMSO. Table 2, on page 18, should be used for making dilutions of the arachidonic acid controls.

Arachidonic Acid Preparation

For the determination of FABP4 binding to arachidonic acid, prepare controls as described below in Table 2. Label seven microcentrifuge tubes S1-S7 and add the indicated amount of ethanol to each tube. Dilute the arachidonic acid stock by adding the appropriate volume from the specified source.

Tube	Arachidonic Acid Concentration (µM)	Arachidonic Acid Source	Arachidonic Acid Volume (µl)	Ethanol (µl)	Arachidonic Acid Assay Concentration (nM)
S1	20	Stock	3.2	196.8	2,000
S2	10	Tube S1	50	50	1,000
S3	2.50	Tube S2	25	75	250
S4	1.00	Tube S3	40	60	100
S5	0.50	Tube S4	50	50	50
S6	0.10	Tube S5	20	80	10
S7	0	N/A	0	100	0

Table 2. Arachidonic Acid dilution instructions

Plate Set Up and Performing the Assay

Refer to pages 11 through 18 to follow the protocols outlined for **Performing the Assay**.

Performance Characteristics

Assay Range:

Under the modified conditions of the assay, the dynamic range for arachidonic acid is 0-2,000 nM. However, this range can be adjusted for experimental samples based on affinity.

Modified Displacement Curve

The plot presented here is an example of the data seen using the modified assay for the displacement of Detection Reagent by Arachidonic Acid; however, your results may vary.

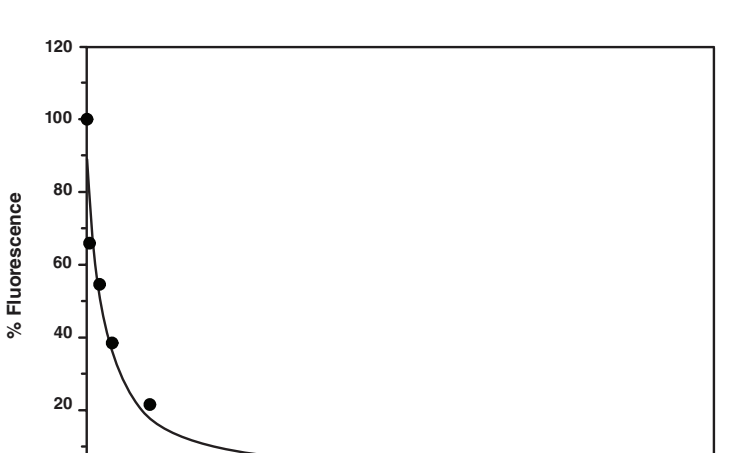
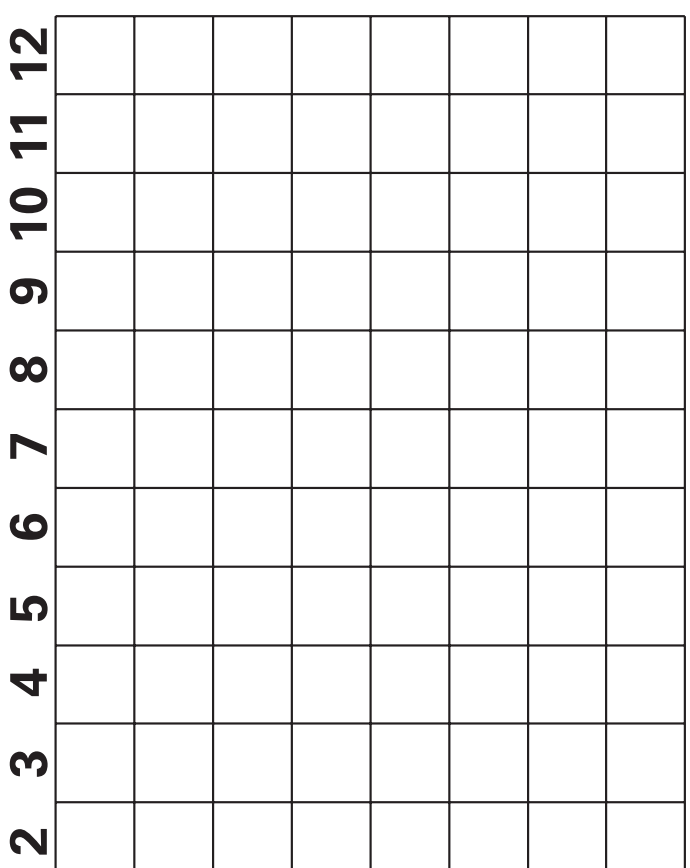


Figure 4. Typical modified displacement curve

References

1. Zimmerman, A.W. and Veerkamp, J.H. New insights into the structure and function of fatty acid-binding proteins. *Cell. Mol. Life Sci.* **59**, 1096-1116 (2002).
2. Massolini, G. and Calleri, E. Survey of binding properties of fatty acid-binding proteins chromatographic methods. *J. Chromatogr. B* **797**, 255-268 (2003).
3. Furuhashi, M., Tuncman, G., Görgün, C.Z., *et al.* Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature* **447**, 959-965 (2007).
4. Boord, J.B., Maeda, K., Makowski, L., *et al.* Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1686-1691 (2002).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.