ab204699

Cyclooxygenase (COX) Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate detection of Cyclooxygenase (COX) activity.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 8b Last Updated 7 October 2024

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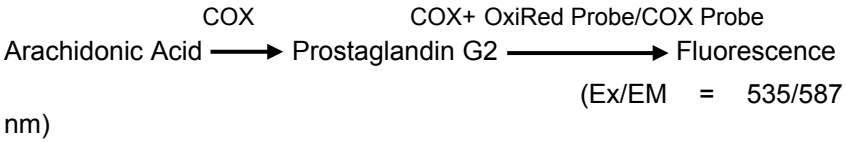
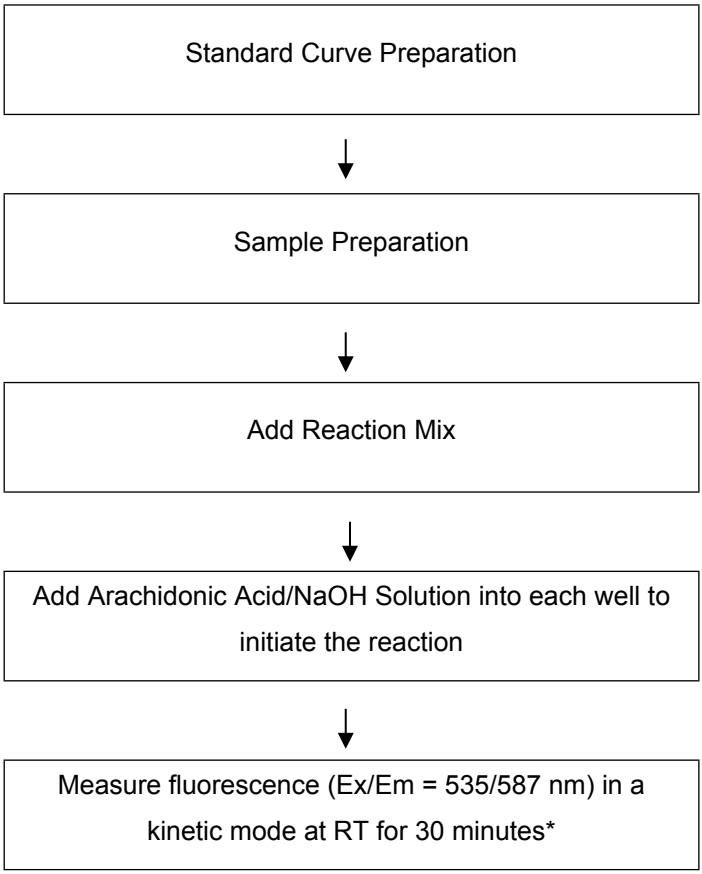
1. BACKGROUND

Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (ab204699) provides a simple, sensitive, and high-throughput adaptable method to detect the peroxidase activity of COX in biological samples or purified/crude enzyme preparations. The kit includes COX-1 and COX-2 specific inhibitors to differentiate the activity of COX-1 and COX-2 as well as other peroxidases, which may be present in the sample. Detection limit: 6 µU/mg.



Cyclooxygenase (COX), (prostaglandin-endoperoxide synthase, PTGS, EC 1.14.99.1), is an enzyme responsible for the formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. COX is the central enzyme in the biosynthetic pathway to prostanoids from arachidonic acid. There are two known isoenzymes: COX-1 and COX-2. COX-1 is constitutively expressed in many tissues and is the predominant form in gastric mucosa and in kidney. COX-2 is not expressed under normal conditions in most cells, but elevated levels are observed during inflammation. Pharmacological inhibition of COX by nonsteroidal anti-inflammatory drugs (NSAID) can provide relief from the symptoms of inflammation and pain.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied 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	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
COX Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/COX Probe (in DMSO)	200 µL	-20°C	-20°C
COX Cofactor/COX Cofactor (in DMSO)	20 µL	-20°C	-20°C
Arachidonic Acid	1 Vial	-20°C	-20°C
NaOH Solution/NaOH	500 µL	-20°C	-20°C
COX-1 Positive Control	1 Vial	-20°C	-80°C
Resorufin Standard/Resorufin Standard (5 mM, in DMSO)	50 µL	-20°C	-20°C
SC560 (COX-1 Inhibitor)/SC560 (COX-1 inhibitor in DMSO)	100 µL	-20°C	-20°C
Celecoxib (COX-2 Inhibitor)/Celecoxib (COX-2 inhibitor in DMSO)	100 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- Cold 1X PBS
- Lysis buffer (e.g.1X PBS with 1% NP40); Protease Inhibitor Cocktail (EDTA-free) (e.g. ab201111)
- DMSO
- Microcentrifuge
- Pipettes and pipette tips
- Multi-channel pipette (adjustable to 10 µL)
- Fluorescent microplate reader – equipped with filter Ex/Em = 535/587 nm
- 96 well plate with clear flat bottom preferably white
- Heat block or water bath
- Dounce homogenizer (if using tissue)

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.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **COX-1 Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **OxiRed Probe/COX Probe (in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot OxiRed Probe/probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Once the OxiRed Probe/probe is thawed, use with two months.

9.3 **NaOH Solution/NaOH:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot NaOH Solution/NaOH so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 **COX Cofactor/COX Cofactor (in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot cofactor so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture.

9.5 **Arachidonic Acid:**

Reconstitute the vial in 55 µl of 100% Ethanol and vortex for 15-30 sec.

9.6 **COX-1 Positive Control:**

Reconstitute with 20 µL of sterile ddH_2O. Aliquot positive control so that you have enough volume to perform the desired number of assays. For short term storage (up to 2 weeks), reconstituted Positive Control can be stored at -20°C. For long term storage, store at -80°C. Keep on ice while in use. Standard is stable for at least 30 minutes on ice.

9.7 **Resorufin Standard/Resorufin Standard (5 mM, in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture.

9.8 **SC560 (COX-1 Inhibitor)/SC560 (COX-1 inhibitor, in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture.

9.9 **Celecoxib (COX-2 Inhibitor)/Celecoxib (COX-2 inhibitor, in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot so that you have enough

volume to perform the desired number of assays. Store at -20°C protected from light and moisture.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare 1 mL of 10 µM (10 pmol/µL) Resorufin standard by diluting 2 µL of the provided 5 mM Resorufin standard with 998 µL of COX Assay Buffer.

10.2 Prepare 500 µL of 1 µM Standard by diluting 50 µL of 10 µM Resorufin standard with 450 µL of COX Assay Buffer.

10.3 Using 1 µM (1 pmol/µL) Resorufin standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc resorufin in well (pmol/well)
1	0	300	100	0
2	12	288	100	4
3	24	276	100	8
4	36	264	100	12
5	48	252	100	16
6	60	240	100	20

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 – 6 x 10^6 cells).

NOTE: *Adherent cells can be scraped off from the culture plate.*

11.1.2 Wash cells with 10 mL cold PBS (1X).

11.1.3 Resuspend in 1 mL PBS (1X) and transfer cells to a 1.5 mL tube.

11.1.4 Centrifuge at 500 x *g* for 3 minutes.

11.1.5 Discard supernatant and resuspend cell pellet in 0.2 – 0.5 mL of lysis buffer with protease inhibitor cocktail.

11.1.6 Vortex and incubate on ice for 5 minutes.

11.1.7 Centrifuge the cell lysate at 12,000 x *g*, 4°C for 3 minutes.

11.1.8 Collect supernatant and keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 50 – 100 mg).

NOTE: *We recommend using perfused tissue samples for preparing tissue homogenates.*

11.2.2 Wash tissue three times with PBS (1X).

11.2.3 Add 0.2 – 0.5 mL of lysis buffer with protease inhibitor cocktail and quickly homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.

11.2.4 Centrifuge the tissue homogenate at 12,000 x *g*, 4°C for 3 minutes.

11.2.5 Collect supernatant and keep on ice.

We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

12.1 Set up standard wells (100 µL). Standard wells can be read in the microplate reader in the end point mode (see Step 12.9).

12.2 Prepare COX Working Solution (200X dilution) by adding 2 µL of COX Cofactor (Step 9.4) to 398 µL of COX Assay Buffer just before use.

NOTE: *Diluted COX Cofactor is stable for 1 hour at room temperature.*

12.3 Prepare Arachidonic Acid Working Solution by adding 5 µL of supplied Arachidonic Acid (Step 9.5) to 5 µL of NaOH Solution/NaOH just before use. Vortex briefly to mix.

Dilute Arachidonic Acid/NaOH solution 10 times further by adding 90 µL ddH_2O, vortex briefly to mix. Make as much as needed (10 µL Arachidonic Acid/NaOH solution/assay). Diluted Arachidonic Acid/NaOH solution is stable for at least 1 hour on in ice.

12.4 **Set up Reaction wells:**

NOTE: *To detect specific activity of COX-1 or COX-2, set up 2 parallel wells.*

- (2x) Sample wells = 2 – 20 µL samples (adjust volume to 20 µL/well with COX Assay Buffer).
- (2x) Positive control = 2 µL positive control + 18 µL COX Assay Buffer.

12.5 Add specific inhibitor to measure COX-1 and/or COX-2 activity as described below:

- **Well 1**: TOTAL COX Activity = 2 µL DMSO.
- **Well 2**: COX-1/COX-2 Activity = 2 µL inhibitor.

To measure COX-1 activity, add COX-1 Inhibitor (SC560) and to measure COX2 activity, add COX-2 Inhibitor (Celecoxib) to the sample well respectively.

Reaction Mix:

Prepare Reaction Mix for the 2 parallel wells:

Component	Reaction Mix (µL)
OxiRed Probe/COX Probe	2
Diluted COX Cofactor	4
COX Assay Buffer	130

Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions + 1)

12.6 Add 68 µL of Reaction Mix into each parallel well in 96-well plate as described in the table below.

	SAMPLE		POSITIVE CONTROL	
Component	TOTAL COX	COX 1 / COX 2	POSITIVE CONTROL	POSITIVE CONTROL + COX-1 Inhibitor
Sample	20 µL	20 µL	-	-
Positive control	-	-	2 µL + 18 µL Assay Buffer	2 µL + 18 µL Assay Buffer
DMSO	2 µL	-	2 µL	-
COX specific inhibitor	-	2 µL	-	2 µL
Reaction Mix	68 µL	68 µL	68 µL	68 µL

12.7 Use a multi-channel pipette to add 10 µL diluted Arachidonic Acid/NaOH Solution into each well to initiate the reaction at the same time.

12.8 After addition of the Arachidonic Acid, measure fluorescence (Ex/Em = 535/587 nm) immediately in a kinetic mode once every 15 sec. for at least 30 minutes.

NOTE: *Preset the plate reader to avoid delay in measurement after addition of Arachidonic Acid/NaOH solution.*

NOTE: *Incubation time depends on sample's COX activity. We recommend measuring fluorescence in a kinetic mode and choosing two time points (T1 and T2) in the linear range to calculate the COX activity of the sample (RFU_S) and sample with inhibitor (RFU_I). The Standard Curve can be read in the end point mode (i.e. at the end of incubation time).*

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Resorufin.

13.4 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Activity of COX is calculated as:

$$\Delta \mathbf{RFU_{535/587nm}} = (\mathbf{RFU_{S2}} - \mathbf{RFU_{S1}}) - (\mathbf{RFU_{I2}} - \mathbf{RFU_{I1}})$$

Where:

RFU_{S1} is the sample reading at time T1.

RFU_{I1} is the inhibitor sample at time T1.

RFU_{S2} is the sample reading at time T2.

RFU_{I2} is the inhibitor sample at time T2.

13.6 Use the $\Delta RFU_{535/587nm}$ to obtain B pmol of resorufin generated by the respective COX isoenzyme during the reaction time ($\Delta T = T_2 - T_1$).

13.7 Activity of COX in the test samples is calculated as:

$$COX\ Activity = \left(\frac{B}{\Delta T x\ M}\right) = pmol/min/mg\ or\ \mu U/mg$$

Where:

B = Amount of resorufin from Standard Curve (pmol).

ΔT = Reaction time (min).

M = Protein amount added into the reaction well (mg)

Note: *To measure COX-1 activity, (RFU_{I2} – RFU_{I1}) is the sample well containing SC560 (COX-1 Inhibitor)/COX-1 Inhibitor (SC560) at T_1 and T_2. To measure COX-2 activity, (RFU_{I2} – RFU_{I1}) is the sample well containing Celecoxib (COX-2 Inhibitor)/COX-2 Inhibitor (Celecoxib) at T_1 and T_2.*

Unit Definition:

1 Unit COX activity = amount of cyclooxygenase which generates 1.0 µmol of resorufin per min. at pH 8.0, 25°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

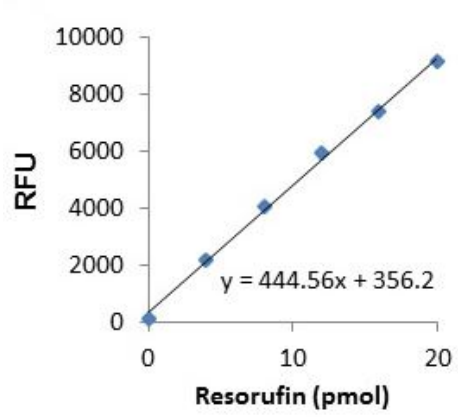


Figure 1. Typical Resorfurin Standard calibration curve.

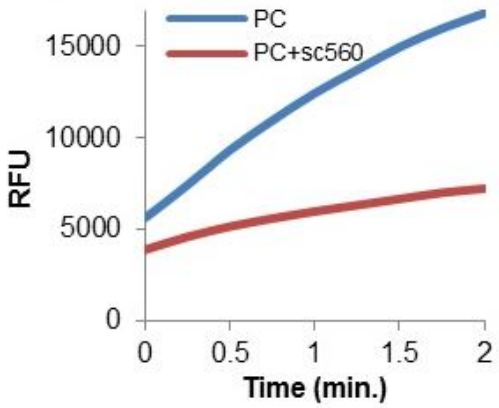


Figure 2. Measurement of COX-1 activity in absence (blue line) and presence of SC560 (COX-1 Inhibitor)/COX-1 inhibitor (SC560, red line) using the COX-1 positive control (PC) provided in the kit.

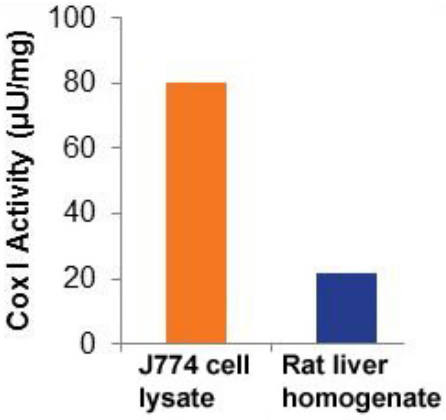


Figure 3. Detection of endogenous COX-1 activity in J774 cell lysate (6 µg) and rat liver homogenate (210 µg).

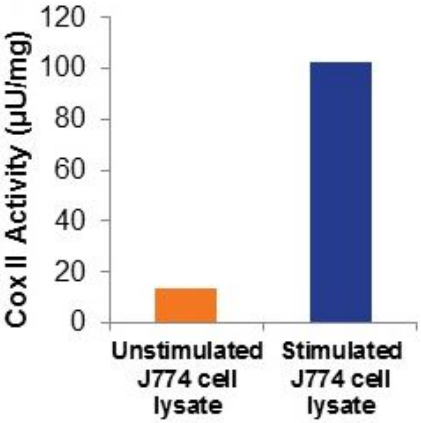


Figure 4. Detection of endogenous COX-2 activity in J774 cell lysate (7 µg) stimulated with or without 100 ng/ml LPS and 100 ng/ml murine IFN-gamma.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, diluted COX cofactor, diluted Arachidonic Acid/NaOH solution and prepare enzyme mix; get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard and sample wells.
- Prepare 2 parallel wells for positive control and each sample for total activity and either COX-1 or COX-2 inhibitor.
- Add DMSO or COX specific inhibitor to each of the parallel wells.
- Prepare COX Reaction Mix (Number samples + positive control + 1).

Component	Reaction Mix (µL)
OxiRed Probe/COX Probe	**2**
Diluted COX Cofactor	**4**
COX Assay Buffer	**130**

- Add 68 µL of COX Reaction Mix to each parallel well.

	SAMPLE		POSITIVE CONTROL	
Component	TOTAL COX	COX 1 / COX 2	POSITIVE CONTROL	POSITIVE CONTROL + COX-1 Inhibitor
Sample	**20 µL**	**20 µL**	**-**	**-**
Positive control	**-**	**-**	**2 µL + 18 µL Assay Buffer**	**2 µL + 18 µL Assay Buffer**
DMSO	**2 µL**	**-**	**2 µL**	**-**
COX specific inhibitor	**-**	**2 µL**	**-**	**2 µL**

Reaction Mix	**68 µL**	**68 µL**	**68 µL**	**68 µL**

- Use a multi-channel pipette to add 10 µL diluted Arachidonic Acid/NaOH Solution into each well to initiate the reaction at the same time.
- Measure fluorescence (Ex/Em = 535/587 nm) immediately in a kinetic mode once every 15 sec. for 30 minutes.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure.

- RIPA buffer – it contains SDS which can destroy/decrease the activity of the enzyme.

18.FAQ

19.NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)