

# **ab133035 – 15(S)-HETE ELISA Kit**

## Instructions for Use

For quantitative detection of 15-hydroxyeicosatetraenoic acid (15(S)-HETE) in plasma, serum, urine, tissue culture media and other biological fluids.

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

# Table of Contents

---

## INTRODUCTION

- 1. BACKGROUND 2
- 2. ASSAY SUMMARY 3

## GENERAL INFORMATION

- 3. PRECAUTIONS 4
- 4. STORAGE AND STABILITY 5
- 5. MATERIALS SUPPLIED 5
- 6. MATERIALS REQUIRED, NOT SUPPLIED 6
- 7. LIMITATIONS 6
- 8. TECHNICAL HINTS 7

## ASSAY PREPARATION

- 9. REAGENT PREPARATION 8
- 10. STANDARD PREPARATIONS 9
- 11. SAMPLE COLLECTION AND STORAGE 11
- 12. PLATE PREPARATION 13

## ASSAY PROCEDURE

- 13. ASSAY PROCEDURE 14

## DATA ANALYSIS

- 14. CALCULATIONS 16
- 15. TYPICAL DATA 17
- 16. TYPICAL SAMPLE VALUES 18
- 17. ASSAY SPECIFICITY 21

## RESOURCES

- 18. TROUBLESHOOTING 22
- 19. NOTES 23

## 1. BACKGROUND

Abcam's 15(S)-HETE *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of 15-hydroxyeicosatetraenoic acid in plasma, serum, urine, tissue culture media and other biological fluids.

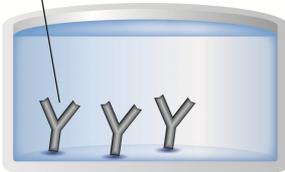
A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-15(S)-HETE antigen and a polyclonal rabbit antibody specific to 15(S)-HETE. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of 15(S)-HETE captured in the plate.

15-hydroxyeicosatetraenoic acid (15(S)-HETE) is the major hydroxy derivative of arachidonic acid when acted upon by 15-lipoxygenase (15-LOX). It is also the primary monohydroxy acid synthesized by the lipoxygenase activity of Cyclooxygenase-1. Aspirin-mediated acetylation of the COX-1 enzyme results in 15(R)-HETE<sub>3</sub>. Blood platelets, peripheral leukocytes, vascular smooth muscle and other cell types produce Type-1 15-LOX while prostate, lung, skin and cornea tissues produce Type-24. 15-HETE has been proposed to act as a paracrine regulator of smooth muscle and lung neutrophil recruitment due in part to its incorporation into tracheal epithelium at the sn-2 position of phosphatidylinositol<sub>6</sub>. The phosphoinositol modification in turn is thought to affect signal transduction and the regulation of intracellular calcium. Interleukin-4 has been shown to regulate 15(S)-HETE expression and incorporation into cellular phospholipids. 15(S)-HETE binds to actin and the alpha-subunit of mitochondrial ATP synthase suggesting a more direct method in regulating some physiological activities. Increased levels of 15(S)-HETE are also

associated with asthma, rhinitis, chronic paranasal sinusitis and rheumatoid arthritis.

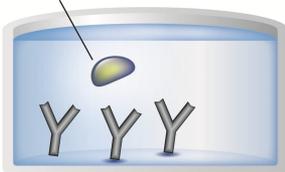
## 2. ASSAY SUMMARY

Capture Antibody



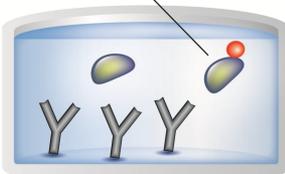
Prepare all reagents and samples as instructed.

Sample



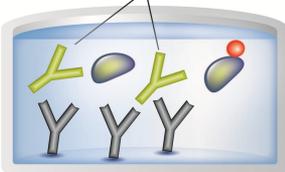
Add standards and samples to appropriate wells.

Labeled AP-Conjugate



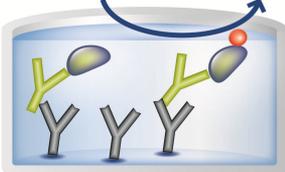
Add prepared labeled AP-conjugate to appropriate wells.

Target Specific Antibody



Add 15(S)-HETE antibody to appropriate wells. Incubate at room temperature.

Substrate      Colored Product



Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

### 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- The activity of the alkaline phosphatase conjugate is dependent on the presence of  $Mg^{2+}$  and  $Zn^{2+}$  ions. The activity of the conjugate is affected by concentrations of chelators ( $>10$  mM) such as EDTA and EGTA
- We test this kit's performance with a variety of samples; however it is possible that high levels of interfering substances may cause variation in assay results
- The 15(S)-HETE Standard provided is supplied in ethanolic buffer at a pH optimized to maintain 15(S)-HETE integrity. Care should be taken handling this material because of the known and unknown effects of eicosanoids

## 4. STORAGE AND STABILITY

Store kit at +4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	+4 °C
15(S)-HETE Alkaline Phosphatase Conjugate	5 mL	-20°C
15(S)-HETE Antibody	5 mL	+4 °C
15(S)-HETE Standard	500 µL	-20°C
Assay Buffer	27 mL	+4 °C
20X Wash Buffer Concentrate	27 mL	+4 °C
pNpp Substrate	20 mL	+4 °C
Stop Solution	5 mL	+4 °C

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

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

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- 200 mg C<sub>18</sub> Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of 15(S)-HETE)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of 15(S)-HETE)
- Deionized water
- Ethanol
- Hexane
- Ethyl acetate

### **7. 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

### 8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- **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**

## 9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

### 9.1 **15(S)-HETE Alkaline Phosphatase Conjugate**

Allow the 15(S)-HETE AP Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

### 9.2 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

## 10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 For:

10.1.1 **Serum/Plasma** samples dilute the 15(S)-HETE standard with Assay Buffer.

10.1.2 **Tissue** culture media samples dilute the 15(S)-HETE standard with tissue culture media.

10.2 Allow the 200,000 pg/mL 15(S)-HETE **Stock Standard** solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.3 Label five tubes with numbers #1 – #5.

10.4 Add 900 µL of appropriate diluent (Assay Buffer or Tissue Culture Media) to tube #1.

10.5 Add 750 µL of appropriate diluent (Assay Buffer or Tissue Culture Media) to tubes #2-#5.

10.6 Prepare a 20,000 pg/mL **Standard 1** by adding 100 µL of the 200,000 pg/mL Stock Standard to tube 1. Vortex thoroughly.

10.7 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Vortex thoroughly.

10.8 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Vortex thoroughly.

10.9 Using the table below as a guide, repeat for tubes 4 and 5.

## ASSAY PREPARATION

Standard	Sample to Dilute	Volume to Dilute ( $\mu\text{L}$ )	Volume of Diluent ( $\mu\text{L}$ )	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	100	900	200,000	20,000
2	Standard 1	250	750	20,000	5,000
3	Standard 2	250	750	5,000	1,250
4	Standard 3	250	750	1,250	312.5
5	Standard 4	250	750	312.5	78.12



## 11. SAMPLE COLLECTION AND STORAGE

- The 15(S)-HETE kit is compatible with 15(S)-HETE samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples containing rabbit IgG may interfere with the assay.
- Culture fluids, serum and plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be small change in binding associated with running the standards and samples in media.
- Users should only use standard curves generated in media or buffer to calculate concentrations of 15(S)-HETE in the appropriate matrix. Plasma, serum and urine samples may need to be extracted. Some samples normally have very low levels of 15(S)-HETE present and extraction may be necessary for accurate measurement. Tissue samples should be extracted. A suitable extraction procedure is outlined below:
  - 11.1. Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50  $\mu$ L of HCl will be needed per mL of plasma. Allow to sit at 4°C for 15 minutes. Centrifuge samples in a micro-centrifuge for 2 minutes to remove any precipitate.
  - 11.2. Prepare the C18 reverse phase column (see Section 6) by washing with 10mL of ethanol followed by 10mL of deionized water.
  - 11.3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and

finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.

- 11.4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250  $\mu\text{L}$  of Assay Buffer to the dried sample. Mix well and hold at room temperature for 5 minutes. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at  $-80^{\circ}\text{C}$  until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

	1	2	3	4
A	B <sub>s</sub>	Std 1	Std 5	Sample 4
B	B <sub>s</sub>	Std 1	Std 5	Sample 4
C	TA	Std 2	Sample 1	Sample 5
D	TA	Std 2	Sample 1	Sample 5
E	NSB	Std 3	Sample 2	etc
F	NSB	Std 3	Sample 2	etc
G	B <sub>0</sub>	Std 4	Sample 3	
H	B <sub>0</sub>	Std 4	Sample 3	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

### Key:

**B<sub>s</sub>** = Blank; contains substrate only.

**TA** = Total Activity; contains conjugate (5 µL) and substrate.

**NSB** = Non-specific binding; contains standard diluent, assay buffer, conjugate and substrate.

**B<sub>0</sub>** = 0 pg/mL standard; contains standard diluent, conjugate, antibody and substrate

## 13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use
- It is recommended to assay all standards, controls and samples in duplicate
- Refer to the recommended plate layout in Section 12 before proceeding with the assay

13.1 Add 100  $\mu$ L appropriate diluent\* into the NSB (non-specific binding) wells. (\*Use the same diluent used to prepare standards in section 10, either Assay Buffer or Tissue Culture Media).

13.1 Add 100  $\mu$ L appropriate diluent (Assay Buffer or tissue culture media) into the B<sub>0</sub> (0 pg/mL standard) wells

13.2 Add 100  $\mu$ L of prepared standards and 100  $\mu$ L diluted samples to appropriate wells

13.3 Add 50  $\mu$ L of Assay Buffer into the NSB wells.

13.4 Add 50  $\mu$ L of 15(S)-HETE Phosphatase Conjugate (blue) into NSB, B<sub>0</sub>, standard and sample wells, i.e. not the Total Activity (TA) and B<sub>s</sub> wells

13.5 Add 50  $\mu$ L of 15(S)-HETE antibody (yellow) into B<sub>0</sub>, standard and sample wells, i.e. not B<sub>s</sub>, TA and NSB wells

**NOTE:** Every well used should be green in color except the NSB wells which should be blue. The B<sub>0</sub> and TA wells are empty at this point and have no color.

13.6 Incubate the plate for 18-24 hours at 4°C. The plate should be covered with the plate sealer.

13.7 Empty the contents of the wells and wash by adding 400  $\mu$ L of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.8 Add 5  $\mu$ L of the 15(S)-HETE Phosphatase Conjugate to the TA wells.

## ASSAY PROCEDURE

- 13.9 Add 200  $\mu\text{L}$  of the pNpp Substrate solution to every well. Incubate at 37°C for 1 hour without shaking.
- 13.10 Add 50  $\mu\text{L}$  Stop Solution into each well. The plate should be read immediately.
- 13.11 Blank the plate reader against the B<sub>s</sub> wells, read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the B<sub>s</sub> wells, manually subtract the mean optical density of the B<sub>s</sub> wells from all readings.

## 14. CALCULATIONS

- 14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}$$

- 14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells ( $B_0$ ), using the following formula

$$\text{Percent Bound} = \frac{\text{Average Net OD}}{\text{Average Net } B_0 \text{ OD}} \times 100$$

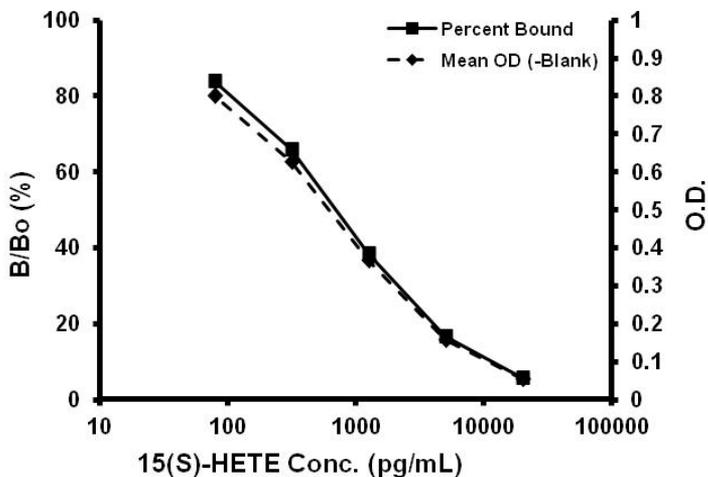
- 14.3 Plot the Percent Bound ( $B/B_0$ ) and the net OD versus concentration of 15(S)-HETE for the standards. The concentration of 15(S)-HETE in the unknowns can be determined by interpolation of net OD values.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor

## 15. TYPICAL DATA

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



Sample	Mean OD (- B <sub>s</sub> )	% Bound	15(S)-HETE <sub>1</sub> (pg/mL)
B <sub>s</sub>	(0.084)		
TA	0.597		
NSB	0.083	0	
B <sub>0</sub>	0.954	100	0
Standard 1	0.056	5.9	20,000
Standard 2	0.159	16.8	5,000
Standard 3	0.367	38.6	1,250
Standard 4	0.628	65.9	312.5
Standard 5	0.802	84.1	78.12
Unknown 1	0.229	24.1	2,872
Unknown 2	0.580	60.8	410

### TYPICAL QUALITY CONTROL PARAMETERS –

Total Activity Added	= $0.598 \times 10 = 5.98$
%B <sub>0</sub> /TA	= 15.97%
Quality of Fit	= 1.000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	= 3,819 pg/mL
50% Intercept	= 710 pg/mL
80% Intercept	= 117 pg/mL

## 16. TYPICAL SAMPLE VALUES

### SENSITIVITY –

The sensitivity, minimum detectable dose of 15(S)-HETE using this 15(S)-HETE ELISA kit was found to be 69.21 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard (Blank), and comparing to the average optical density for Standard 5. The detection limit was determined as the concentration of 15(S)-HETE measured at two standard deviations from the zero along the standard curve.

### SAMPLE RECOVERY –

Recovery was determined by 15(S)-HETE in tissue culture media, human urine, porcine EDTA plasma and porcine serum. Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	100.3	None
Human Urine	89.6	> 1:8
Porcine EDTA Plasma	105.4	1:4 – 1:32
Porcine Serum	95.9	>1:16

### LINEARITY OF DILUTION –

A sample containing 9,923 pg/mL 15(S)-HETE was serially diluted 5 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual 15(S)-HETE concentration versus measured 15(S)-HETE concentration.

The line obtained had a slope of 0.8184 and a correlation coefficient of 0.99927

## PRECISION –

### Intra-Assay

	<b>15(S)-HETE (pg/mL)</b>	<b>%CV</b>
Low	181	15.8
Medium	853	9.8
High	3,929	3.4

### Inter-Assay

	<b>15(S)-HETE (pg/mL)</b>	<b>%CV</b>
Low	192	19.1
Medium	730	8.9
High	4,335	6.1

**17. ASSAY SPECIFICITY****CROSS REACTIVITY –**

The cross reactivities for a number of related eicosanoid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 500,000 to 5 pg/mL. These samples were then measured in the 15(S)-HETE assay, and the measured 15(S)-HETE concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

15(S)-HETE	100%
5,15-diHETE	1.0%
8,15-diHETE	1.0%
13(S)-HODE	0.6%
5-HETE	0.1%
PGB <sub>2</sub>	0.1%
PGD <sub>2</sub>	0.1%
PGF <sub>2</sub> alpha	0.1%
12(S)-HETE	<0.05%
12(R)-HETE	<0.05%
Arachidonic Acid	<0.05%
PGE <sub>2</sub>	<0.05%
Linoleic Acid	<0.05%

## 18. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES







**UK, EU and ROW**

Email: [technical@abcam.com](mailto:technical@abcam.com) | Tel: +44-(0)1223-696000

**Austria**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 019-288-259

**France**

Email: [supportscientifique@abcam.com](mailto:supportscientifique@abcam.com) | Tel: 01-46-94-62-96

**Germany**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 030-896-779-154

**Spain**

Email: [soportecientifico@abcam.com](mailto:soportecientifico@abcam.com) | Tel: 911-146-554

**Switzerland**

Email: [technical@abcam.com](mailto:technical@abcam.com)

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

**US and Latin America**

Email: [us.technical@abcam.com](mailto:us.technical@abcam.com) | Tel: 888-77-ABCAM (22226)

**Canada**

Email: [ca.technical@abcam.com](mailto:ca.technical@abcam.com) | Tel: 877-749-8807

**China and Asia Pacific**

Email: [hk.technical@abcam.com](mailto:hk.technical@abcam.com) | Tel: 400 921 0189 / +86 21 2070 0500

**Japan**

Email: [technical@abcam.co.jp](mailto:technical@abcam.co.jp) | Tel: +81-(0)3-6231-0940

[www.abcam.com](http://www.abcam.com) | [www.abcam.cn](http://www.abcam.cn) | [www.abcam.co.jp](http://www.abcam.co.jp)