ab65341
Free Fatty Acid Quantification Assay Kit (Colorimetric/ Fluorometric)

Instructions for use:

For rapid, sensitive and accurate measurement of Free Fatty Acid in various samples.

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

Version 13 Last Updated: 25 May 2016

Table of Contents

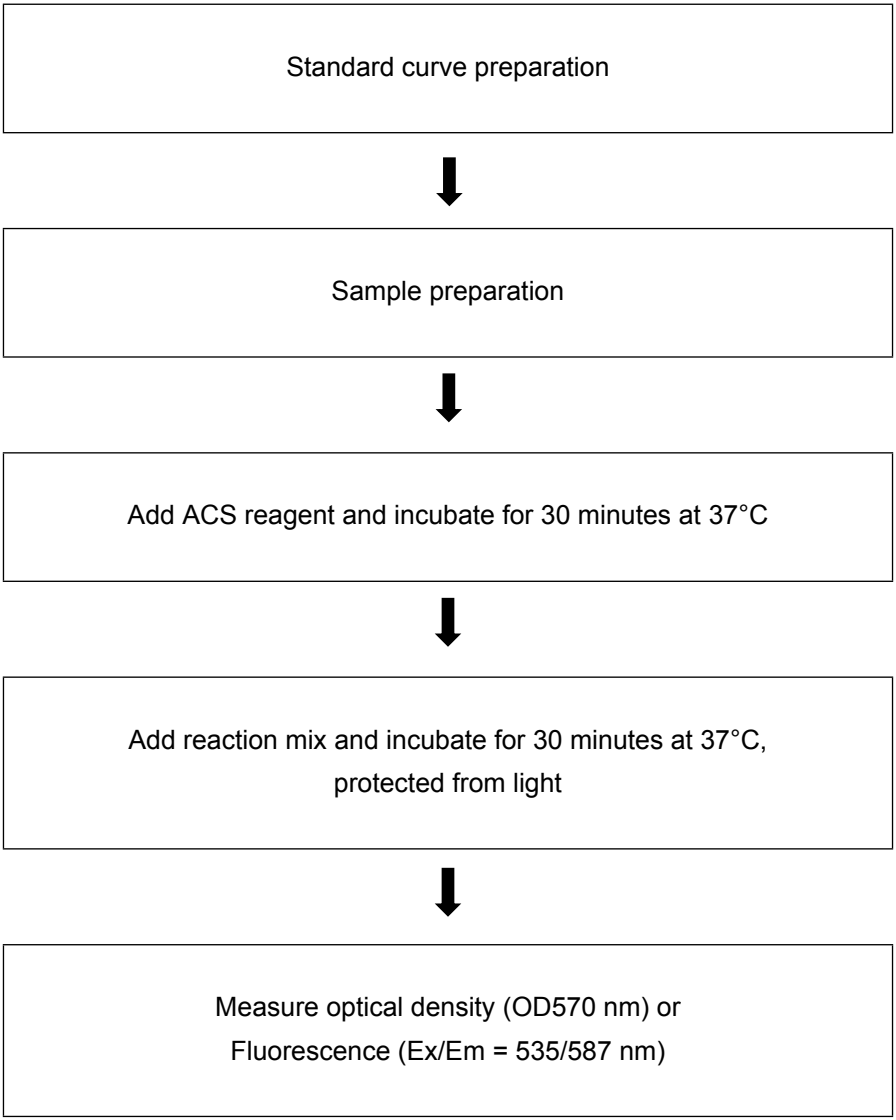
1. BACKGROUND

Free Fatty Acid Quantification Assay Kit (Colorimetric/Fluorometric) (ab65341) provides a convenient, sensitive enzyme-based method for detecting the long-chain free fatty acids in various biological samples, such as serum, plasma and other body fluids, food or growth media.

In this assay, Fatty Acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of color or fluorescence. C-8 (octanoate) and longer fatty acids can then be easily quantified by either colorimetric (spectrophotometry at λ = 570 nm) or fluorometric (at Ex/Em = 535/587 nm) methods with detection limit 2 µM free fatty acid in variety samples.

Fatty Acids play very important roles in normal metabolism and many disease developments. They are precursors to a number of bioactive classes of compounds such as prostaglandins, leucotrienes and others, and have been implicated in diverse functions such as autism, immune system and inflammation response.

2. ASSAY SUMMARY



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.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at -20°C 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 the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

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)
Fatty Acid Assay Buffer	25 mL	-20°C	-20°C
Fatty Acid Probe (in DMSO)	200 µL	-20°C	-20°C
Acyl-CoA Synthetase (ACS) Reagent	1 vial	-20°C	-20°C
Enzyme Mix	1 vial	-20°C	-20°C
Enhancer	200 µL	-20°C	-20°C
Palmitic Acid Standard (1nmol/µL)	300 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:

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (colorimetric assay) / 96 well plate with clear flat bottom, preferably black (fluorometric assay)
- Dounce homogenizer (if using tissue)
- Triton X-100
- Chloroform: chloroform is potentially hazardous, so please use in a well ventilated area or fume hood.
- Vacuum dryer

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 all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **Fatty Acid Assay Buffer:**

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

9.2. **Fatty Acid Probe:**

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 let at room temperature, so it needs to melt for few minutes at 37°C.* Keep on ice during the assay. Aliquot probe so that you have enough volume to performed the desired number of assays. Store aliquots at -20°C protected from light and moisture. Use within two months.

9.3. **Acyl-CoA Synthetase (ACS) Reagent:**

Reconstitute the ACS Reagent with 220 µL Assay Buffer. Keep on ice during the assay. Aliquot reagent so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. Use within two months.

9.4. **Enzyme Mix:**

Reconstitute the Enzyme Mix with 220 µL Assay Buffer. Keep on ice during the assay. Aliquot mix so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. Use within two months.

9.5. **Enhancer:**

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

9.6. **Palmitic Acid Standard (1 nmol/µL):**

Frozen storage may cause the Palmitic Acid Standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed, place in a hot water bath (~80-100°C) for 1 minutes or until the standard looks cloudy, vortex for 30 seconds; the standard should become clear. Repeat the heat and vortex one more time. The Palmitic Acid Standard is now completely in solution and ready to use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Discard the working standard dilutions after use as they do not store well.

10.1. **For colorimetric assay:**

10.1.1. Using the Palmitic Acid Standard (1 nmol/µL), prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Palmitic Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Palmitic acid Conc in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2. **For the fluorometric assay:**

10.2.1. Prepare a 0.1 nmol/µL Palmitic Acid standard by diluting 20 µL of the 1 nmol/µL Standard with 180 µL of Assay Buffer.

10.2.2. Using 0.1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Palmitic Acid Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Palmitic acid Conc in well (nmol/well)
1	0	150	50	0
2	6	144	50	0.2
3	12	138	50	0.4
4	18	132	50	0.6
5	24	126	50	0.8
6	30	120	50	1

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

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 your samples in liquid nitrogen upon extraction and store them 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.
- Chloroform is a potentially hazardous component, so please use in a well ventilated area or fume hood.

11.1. **Cell (adherent or suspension) samples:**

11.1.1. Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2. Wash cells with cold PBS.

11.1.3. Homogenize cells in 200 µL chloroform/Triton X-100 (1% Triton X-100 in pure chloroform) by pipetting up and down or using a micro-homogenizer. Incubate on ice 10 – 30 minutes.

11.1.4. Spin the extract for 5 – 10 minutes at top speed in a microcentrifuge.

11.1.5. Collect organic phase (lower phase), air dry at 50°C in a fume hood to remove chloroform.

11.1.6. Vacuum dry for 30 minutes to remove trace chloroform.

11.1.7. Dissolve the dried lipids in 200 µL of Fatty Acid Assay Buffer by vortexing extensively for 5 minutes. ***NOTE:*** *The solution may be slightly turbid or opalescent, but this does not affect the assay.*

11.2. **Tissue samples:**

11.2.1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg tissue).

11.2.2. Wash tissue in cold PBS.

11.2.3. Homogenize in 200 µL of chloroform/Triton X-100 (1% Triton X-100 in pure chloroform) in a micro-homogenizer or Dounce homogenizer. Incubate on ice 10 – 30 minutes.

11.2.4. Centrifuge the extract for 5 – 10 minutes at top speed using a microcentrifuge.

11.2.5. Collect the organic phase (lower phase), air dry at 50°C to remove chloroform in a fume hood.

11.2.6. Vacuum dry for 30 minutes to remove trace chloroform.

11.2.7. Dissolve the dried lipids in 200 µL of Fatty Acid Assay Buffer by vortexing extensively for 5 minutes. ***NOTE:*** *The solution may be slightly turbid or opalescent, but this does not affect the assay.*

11.3. **Liquid Samples (plasma, serum, urine and other biological fluids):**

Liquid samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample in a range (1/2 – 1/200).

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

The extraction procedure can be proportionally scaled up if larger amount of sample is desired.

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **We recommend that you 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 Reaction wells:**

Standard wells = 50 µL Standard dilutions.

Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2. Add 2 µL of ACS Reagent into all standard and sample wells.

12.3. Mix and incubate the reaction for 30 minutes at 37°C.

12.4. **Reaction Mix (COLORIMETRIC ASSAY):**

12.4.1. Prepare 50 µL Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) 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).

Component	Reaction Mix (µL)
Assay Buffer	44
Fatty Acid Probe	2
Enzyme Mix	2
Enhancer	2

12.4.2. Add 50 µL of Reaction Mix to each well.

12.4.3. Incubate at 37ºC for 30 minutes protected from light.

12.4.4. Measure output immediately on a microplate reader at OD 570 nm.

12.5. **Reaction Mix(FLUOROMETRIC ASSAY):**

12.5.1. Prepare 50 µL Reaction Mix for each reaction: Mix enough reagents for the number of assays (samples and controls) 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).

Component	Reaction Mix (µL)
Assay Buffer	45.6
Fatty Acid Probe*	0.4
Enzyme Mix	2
Enhancer	2

***NOTE**: For fluorometric readings, using 0.4 µL/well of the Fatty Acid probe decreases the background readings, therefore increasing detection sensitivity.*

12.5.2. Add 50 µL of Reaction Mix to each well.

12.5.3. Incubate at 37ºC for 30 minutes protected from light.

12.5.4. Measure output immediately on a microplate reader at Ex/Em= 535/587 nm.

13. CALCULATIONS

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

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 Fatty Acid.

13.4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5. Concentration of Free Fatty Acid in the test samples is calculated as:

$$Fatty\ Acid\ Concentration = \left(\frac{Fa}{Sv}\right) * D$$

Where:

Fa = amount of fatty acid in the sample well calculated from standard curve (nmol).

Sv = amount of sample volume added in sample wells (µL).

D = sample dilution factor.

14. TYPICAL DATA

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

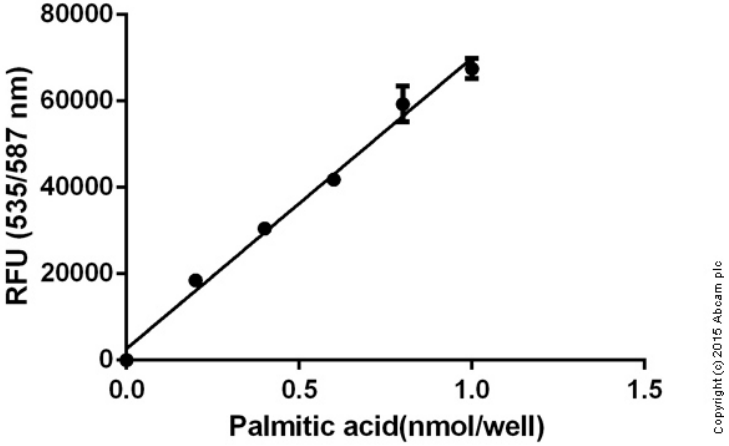


Figure 1: Colorimetric standard curve: mean of duplicates (+/-SD) with background readings subtracted

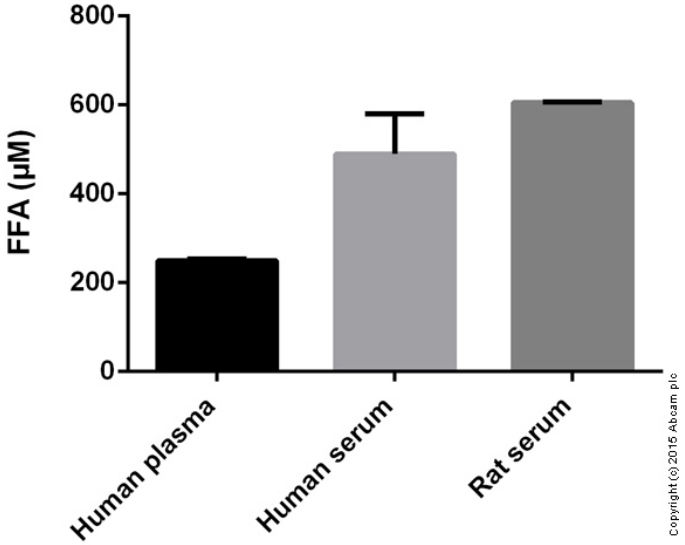


Figure 2: Free Fatty Acid measured in biologicals showing concentration (µM).

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.*

- Solubilize the Free Fatty Acid standard, thaw Fatty Acid probe, prepare enzyme mix and ACS Reagent (aliquot if necessary); get equipment ready.
- Prepare Fatty Acid standard dilution for your desires detection method: colorimetric [2 – 10 nmol/well] or fluorometric [0.2 – 1 nmol/well].
- Prepare samples in optimal dilutions so that they fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL).
- Add 2 µL ACS Reagent to standard and sample wells, mix, and incubate at 37°C for 30 minutes.
- Prepare a master mix for Free Fatty Acid Reaction Mix:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer	44	45.6
Fatty Acid Probe	2	0.4
Enzyme Mix	2	2
Enhancer	2	2

- Add 50 µL Reaction Mix into standard and sample wells.
- Incubate plate at 37°C for 30 minutes protected from light.
- Measure plate at OD 570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

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 provided 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 described in the 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:

- Avoid heparin when preparing plasma or serum samples.

18. FAQ

Which anticoagulants interfere with this assay and which can be used?

Blood needs to be collected using an anticoagulant such as EDTA, sodium citrate, sodium fluoride, or ammonium oxalate. Heparinized plasma is not the best choice as high amounts of heparin could interfere with the assay.

What is the concentration of fatty acid in human serum?

Normal human serum has 80-250 µg/mL Fatty acid.

Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use “media only” as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

What is the length of fatty acid chains that can be quantified using this kit?

A palmitic acid standard is used, but oleic and stearic acid chains can be detected as well.

What is the detection limit for this kit?

The detection limit is ~2 µM fatty acid.

Can the lipid extraction solution from ab102513 (Adipogenesis Detection Kit) be used for cells/tissues in this kit?

Homogenization in 1% Triton X-100 in pure chloroform is much more effective than the Lipid extraction solution from ab102513 and hence is not recommended. Moreover, the chloroform is dried in this assay to get lipids which is not possible with the lipid extraction solution.

How long should samples be air-dried?

Typically up to 1 hour is enough to air- dry at 50°C to remove chloroform. The goal to remove as much chloroform at this step as possible.

Can protein content be used as an internal control for this assay?

Since the tissues/cells are homogenized in chloroform/Triton, a protein assay is not recommended.

Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, snap-frozen samples in liquid nitrogen can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple times (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

19. NOTES

RESOURCES

RESOURCES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

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