ab156915 – Universal SIRT Activity Assay Kit (Colorimetric)

Instructions for Use

For the measurement of activity of total SIRT enzymes using nuclear extracts or purified SIRT isoforms (SIRTs1-7) in various samples

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

Version 6b Last Updated 3 October 2023

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1. BACKGROUND

Acetylation of the epsilon amino group of specific lysine residues contained in core histones is one of the most robust epigenetic marker and is essential for the regulation of multiple cellular processes. The acetylation of histone by histone acetyltransferases (HAT) seems to be of particular significance, as it is associated with active regions of the genome. In contrast, histone deacetylation by histone deacetylase (HDAC) leads to transcription repression. So far, at least 4 classes of HDACs have been identified. Class I HDACs include 1, 2, 3 and 8. Class II HDACs are comprised of 4, 5, 6, 7, 9 and 10. Class III enzymes, known as the sirtuins, require NAD+ cofactors and include SIRTs 1-7. Class IV enzymes, which contains only HDAC11, has features of both Class I and II.

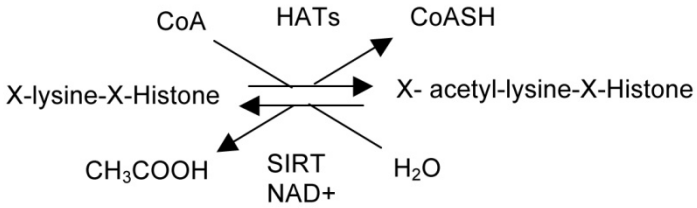


Figure 1. Histone acetylation and deacetylation catalyzed by HATs and HDACs

Unlike other known protein deacetylases, which simply hydrolyzeacetyl-lysine residues, sirtuins catalyze a reaction that couples lysine deacetylation to NAD hydrolysis, yielding O-acetyl-ADP-ribose and nicotinamide. Sirtuins have been implicated in influencing aging and regulating transcription, apoptosis, and stress resistance, as well as energy efficiency and alertness during low-calorie situations. SIRTs are also involved in the development of human diseases including cancer, diabetes, and various neurological diseases. For example, in prostate cancer, SIRT1 was found to be overexpressed. It was also observed that SIRTs protect neurons in Alzheimer's disease. Detection of inhibition or activation of SIRTs would be important in elucidating mechanisms of epigenetic regulation of gene

activation and silencing and benefiting diagnostics and therapeutics of cancer or neurological diseases.

There are only a couple of methods used for detecting SIRT activity/inhibition. These methods are based on the measurement of the deacetylated histone cleavage by lysyl endopeptidase or trypsin, and have significant weaknesses: (1) nuclear extracts from cell/tissues cannot be used because of interfering by lysyl endipeptidases from extracts; (2) Trypsin-sensitive SIRT inhibitiors or activators are not suitable for testing with these methods, as trypsin digestion can lead to false positives when trypsin inhibitors or activators present in the compound library; (3) High interference by DMSO and thiol-containing chemicals, which are often contained in enzyme solutions or tested compound solvents; and (4) Less accurate than a direct measurement of SIRT-converted deacetylated products. We have now added the EpiSeeker Universal SIRT Activity Assay Kit (Colorimetric) to address this issue.

This kit has the following advantages and features:

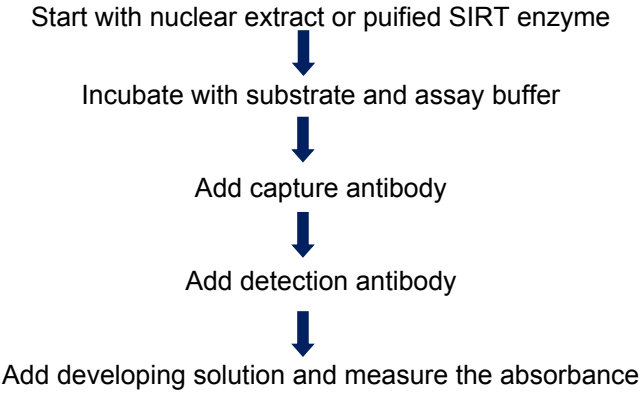
- Colorimetric assay with easy-to-follow steps for convenience and speed. The entire procedure can be finished within 5 hours.
- Strip microplate format makes the assay flexible and quick: manual or high throughput analysis can be completed within 3.5 hours.
- Unique kit composition enables background signals to be very low, which allows the assay to be accurate, sensitive, reliable, and consistent. Innovative colorimetric assay measures SIRT activity/inhibition by directly detecting SIRT-converted deacetylated products, rather than trypsin-based peptide cleavage, thus eliminating assay interference caused by DMSO and thiol-containing chemicals, trypsin, and cellular lysyl endipeptidases.
- Both cell/tissue extracts and purified SIRT enzymes can be used, which allows for the detection of inhibitory effects of SIRT inhibitor in vivo and in vitro.
- Novel assay principle allows high sensitivity to be achieved. The activity can be detected from as low as 1 ng of purified SIRT

enzyme, which is about 10 fold higher than that obtained by trypsin-based peptide cleavage assays.

- A deacetylated histone standard is included, which allows for the specific activity of SIRTs to be quantified.
- Nicotinamide, a SIRT inhibitor as the positive inhibition control, and trichostatin A, an inhibitor of HDACI/II used to block HDAC activity, are both included.

The Universal SIRT Activity Assay Kit (Colorimetric) (ab156915) is suitable for measuring activity/inhibition of total SIRT enzyme using nuclear extracts or purified SIRT isoforms (SIRTs1-7) from a broad range of species such as mammals, plants, fungi, and bacteria, in a variety of forms including, but not limited to cultured cells, fresh and frozen tissues. For SIRT1, SIRT6, and SIRT7, nuclear extracts are required and can be prepared by using your own successful method. For your convenience and the best results, Abcam offers a nuclear extraction kit (ab113474) optimized for use with this kit. Nuclear extracts can be used immediately or stored at –80°C for future use. For SIRT2, SIRT4, and SIRT5, cytoplasmic extracts are required. For SIRT3, mitochondria fractions should be used. Purified enzymes can be active SIRTs from recombinant proteins or isolated from cell/tissues.

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

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt away from light.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

Check if a blue color is present in Developing Solution, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of Developing Solution required into a secondary container (tube or vial) before adding Developing Solution into the assay wells.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	14 mL	28 mL	4°C
SIRT Assay Buffer	4 mL	8 mL	RT
SIRT Substrate, 50X	60 µL	120 µL	-20°C
SIRT Assay Standard, 50 µg/mL	10 µL	20 µL	-20°C
Capture Antibody, 1000X	5 µL	10 µL	4°C
Detection Antibody, 2000X	6 µL	12 µL	-20°C
SIRT Co-factor, 50X	50 µL	100 µL	-20°C
HDAC inhibitor, 50 µM	50 µL	100 µL	-20°C
SIRT Inhibitor, 50 mM	40 µL	80 µL	-20°C
Developer Solution	5 mL	10 mL	4°C
Stop Solution	5 mL	10 mL	RT
8-Well Assay Strips (With Frame)	6	12	4°C
Adhesive Covering Film	1	1	RT

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Microplate reader capable of reading absorbance at 450 nm
- 1.5 mL microcentrifuge tubes
- Incubator for 37°C incubation
- Distilled water
- Nuclear extracts or purified enzymes
- Parafilm M or aluminum foil

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- 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
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- 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.
- Complete removal of all solutions and buffers during wash steps.
- **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

9.1. **1X Wash Buffer**

48-Assay Kit:

Add 13 mL of 10X Wash Buffer to 117 mL of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit:

Add 26 mL of 10X Wash Buffer to 234 mL of distilled water and adjust pH to 7.2-7.5.

This diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2. **Capture Antibody**

Dilute Capture Antibody with 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µL of Capture Antibody to 1000 µL of 1X Wash Buffer). 50 µL of Diluted Capture Antibody will be required for each assay well.

9.3. **Detection Antibody**

Dilute Detection Antibody with 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µL of Detection Antibody to 2000 µL of 1X Wash Buffer). 50 µL of Diluted Detection Antibody will be required for each assay well

9.4. **SIRT Standard**

Suggested Standard Curve Preparation: Dilute SIRT Standard with Assay Buffer 5 ng/µL by adding 1 µL of SIRT Assay Standard to 9 µL of Assay Buffer. Then, further prepare five concentrations by combining the 5 ng/µL diluted SIRT Standard with Assay Buffer into final concentrations of 0.2, 0.5, 1, 2, and 5 ng/µL according to the following dilution chart:

Tube	SIRT Standard (µL)	Assay Buffer (µL)	Final Conc (ng/µL)
1	1	24	0.2
2	1	9	0.5
3	1	4	1.0
4	2	3	2.0
5	4	0	5.0

***Note**: Keep each of diluted solutions except 1X Wash Buffer on ice until use. Any remaining diluted solutions other than 1X Wash Buffer should be discarded if not used within the same day.*

Suggested Buffer and Solution Setup

Approximate amount of required buffers and solutions for defined assay wells based on the protocol

Reagents	1 well	1 Strip (8 wells)	2 Strip (16 wells)	6 Strip (48 wells)	12 Strip (96 wells)
1X Wash Buffer	2.5 mL	20 mL	40 mL	120 mL	240 mL
Assay Buffer	50 µL	400 µL	800 µL	2400 µL	4800 µL
SIRT Substrate	1 µL	8 µL	16 µL	50 µL	120 µL
SIRT Standard	NA	NA	1 µL	2 µL	2 µL
Diluted Capture Antibody	50 µL	400 µL	800 µL	2400 µL	4800 µL
Diluted Detection Antibody	50 µL	400 µL	800 µL	2400 µL	4800 µL
SIRT Co-factor	1 µL	16 µL	32 µL	96 µL	192µL
Developer Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
Stop Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL

10. SAMPLE PREPARATION

Input Amount: The amount of nuclear extracts for each assay can be between 0.5 µg to 20 µg with an optimal range of 5-10 µg. The amount of purified enzymes can be 5 ng to 500 ng, depending on the purity and catalytic activity of the enzymes.

Nuclear Extraction: You can use your method of choice for preparing nuclear extracts. Abcam offers a Nuclear Extraction Kit (ab113474) optimized for use with this kit. Cell extract or purified SIRT enzyme should be stored in aliquot at –80ºC until use.

11. PLATE PREPARATION

The suggested strip-well plate setup for quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	NNC	NNC	Sample	Sample	Sample	Sample
C	SIRT standard 0.2 ng	SIRT standard 0.2 ng	Sample	Sample	Sample	Sample
D	SIRT standard 0.5 ng	SIRT standard 0.5 ng	Sample	Sample	Sample	Sample
E	SIRT standard 1 ng	SIRT standard 1 ng	Sample	Sample	Sample	Sample
F	SIRT standard 2 ng	SIRT standard 2 ng	Sample	Sample	Sample	Sample
G	SIRT standard 5 ng	SIRT standard 5 ng	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

12. ASSAY PROCEDURE

11.1 **Enzymatic Reaction**

11.1.1 Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

11.1.2 Blank Wells: Add 48 µL of SIRT Assay Buffer,1 µL of SIRT Substrate and 1 µL of SIRT Co-factor. Total volume should be 50 µL/well.

11.1.3 No SIRT Co-factor Control (NNC) Wells: Add 44 to 47 µL of SIRT Assay Buffer, 1 µL of SIRT Substrate, 1 µL of HDAC Inhibitor, and 1 to 4 µL of nuclear extracts or 1 to 4 µL of purified SIRT enzyme. Total volume should be 50 µL/well.

11.1.4 Standard Wells: Add 49 µL of SIRT Assay Buffer and 1 µL of Diluted SIRT Standard to each standard well with a minimum of five wells, each at different concentrations between 0.2 to 5 ng/µL (based on the dilution chart in 9.4); see Table as an example).

11.1.5 Sample Wells Without Inhibitor: Add 42 to 45 µL of SIRT Assay Buffer, 1 µL of SIRT Substrate, 1 µL of HDAC Inhibitor, 1 µL of SIRT Co-factor, and 1 to 4 µL of nuclear extracts or 1 to 4 µL of purified SIRT enzyme. Total volume should be 50 µL/well.

11.1.6 Sample Wells With Inhibitor: Add 36 to 39 µL of SIRT Assay Buffer, 1 µL of SIRT Substrate, 1 µL of HDAC Inhibitor, 1 µL of SIRT Co-factor, and 1 to 4 µL of nuclear extracts or 1 to 4 µL of purified HDAC enzyme, and 5 µL of inhibitor solution. Total volume should be 50 µL/well.

Note: *(1) Follow the suggested well setup in Table 1; (2) It is recommended to use 2 µg to 10 µg of cell extract per well or 10 ng to 200 ng of purified enzyme per well; (3) The concentration of inhibitors to be added into the sample wells can be varied (e.g., 1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with SIRT Assay Buffer at a 1:10 ratio (e.g., add 0.5 µL of inhibitor to*

4.5 µL of SIRT Assay Buffer), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less. The SIRT inhibitor, nicotinamide included in the kit can be used as a control inhibitor.

11.1.7 Tightly cover strip plate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60-90 min.

Note: *(1) The incubation time may depend on intrinsic SIRT activity. However, in general, 60 min incubation is suitable for active purified SIRT enzyme and 90 min incubation is required for nuclear extract; (2) The Adhesive Covering Film can be cut to the required size to cover the strips based on the number of strips to be used.*

11.1.8 Remove the reaction solution from each well. Wash each well three times with 150 µL of 1X Wash Buffer each time.

11.2 **Antibody Binding and Signal Enhancing**

11.2.1 Add 50 µL of the Diluted Capture Antibody to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.

11.2.2 Remove the Diluted Capture Antibody solution from each well.

11.2.3 Wash each well three times with 150 µL of the 1X Wash Buffer each time.

11.2.4 Add 50 µL of the Diluted Detection Antibody to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

11.2.5 Remove the Diluted Detection Antibody solution from each well.

11.2.6 Wash each well four times with 150 µL of the 1X Wash Buffer each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step. The wash can be done by simply pipetting the washing buffer into the wells and then pipetting the buffer out from the wells (discard the buffer).*

11.3 **Signal Detection**

11.3.1 Add 100 µL of Developer Solution to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color changes in the sample wells and control wells. The Developer Solution will turn blue in the presence of sufficient hydroxymethylated DNA.

11.3.2 Add 100 µL of Stop Solution to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice - once at 450 nm and once at 655 nm. Then manually subtract the 655 nm OESR10 from 450 nm ODs; (2) If the stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

13. ANALYSIS

12.1 **SIRT Activity Calculation**

Calculate the average duplicate readings for sample wells and blank wells. Calculate SIRT activity or inhibition using the following formulas:

Simple Calculation:

$$[SIRT\ Activity\ (OD/min/mg)] = \left(\frac{Sample\ OD - NNC\ OD}{Protein\ Amount\ (\mu g)\ *\ \times\ min\ **}\right) \times 1000$$

* Protein amount added into the reaction at step 11.1.5
**Incubation time at step 11.1.7 (in minutes).

Example calculation:
Average OD450 of sample is 0.75
Average OD450 of NNC is 0.15
Protein amount is 5 µg
Incubation time is 2 hours (120 min)

$$SIRT\ Activity\ = \frac{0.75 - 0.15}{5 \times 120} \times 1000\ = 1\ OD/min/mg$$

For accurate or specific activity and inhibition calculation

First, generate a standard curve and plot OD value versus amount of SIRT Standard at each concentration point. Then, determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), and then calculate the amount of SIRT-converted deacetylated product using the following formulas:

$$[Deacetylated\ Product\ (ng)] = \left(\frac{Sample\ OD - NNC\ OD}{Slope}\right)$$

$$SIRT\ Activity\ (ng/min/mg) = \left(\frac{Deacetylated\ Product\ (ng)}{Protein\ Amount\ (\mu g)\ x\ min\ *}\right) \times 1000$$

For inhibition calculation

$$Inhibition\ \% = 1 - \left(\frac{Inhibitor\ Sample\ OD - NNC\ OD}{No\ Inhibitor\ Sample\ OD - NNC\ OD}\right) \times 100\%$$

12.2 Typical Results

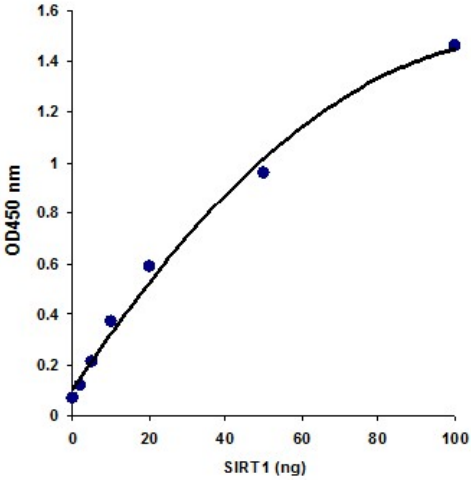


Figure 1. Demonstration of high sensitivity of a SIRT activity assay achieved by using recombinant SIRT1 with Universal SIRT Activity Assay Kit (Colorimetric).

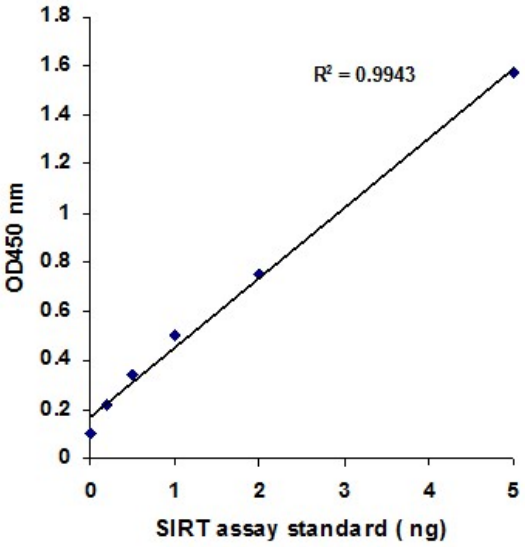


Figure 2. Illustrated standard curve generated with SIRT assay standard.

14. TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake
	The well is incorrectly washed before enzyme reaction	Ensure the well is not washed prior to adding the positive control and sample
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol is followed correctly
	Incorrect absorbance reading	Check if appropriate absorbance wavelength (450 nm) is used
	Kit was not stored or handled properly	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use

No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 11.1.3	Ensure a sufficient amount of standard is added
	The standard is degraded due to improper storage conditions	Follow the Shipping & Storage guidance in this User Guide for storage of SIRT Assay Standard
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips
	Incubation time with Diluted ESR6 is too long.	The incubation time at Step 11.2.4 should not exceed 2 hours
	Over-development of color.	Decrease the development time in Step 11.3.1 before adding Stop Solution in Step 11.3.2

No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified	Ensure your protocol is suitable for SIRT protein extraction. For the best results, it is advised to use Nuclear Extraction Kit (ab113474). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity
	Sample amount added into the wells is insufficient	Ensure a sufficient amount of purified enzymes or nuclear extracts is used as indicated in Step 11.1. The sample can be titrated to determine the optimal amount to use in the assay
	Sample was not stored properly or has been stored for too long	Ensure sample is stored in aliquots at –80°C, with no more than 6 weeks for nuclear extracts and 6 months for purified enzymes. Avoid repeated freezing/thawing
	Little or no activity of SIRT contained in the sample	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts or purified enzymes

Uneven color development	Insufficient washing of the wells	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12)

15. 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)