Version 4 Last updated 3 June 2020

# ab221011 p38 MAPKalpha (pT180/Y182) SimpleStep ELISA® Kit

For the semi-quantitative measurement of p38 MAPKα (pT180/Y182) in Human and mouse cell lysates.

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

# Table of Contents

# 1. Overview

p38 MAPKα (pT180/Y182) in vitro SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of p38 MAPKα (pT180/Y182) protein in Human and mouse cells.

The SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or controls are added to the wells, followed by the Antibody Cocktail. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

p38 MAPKα is a member of the MAP kinase family, which are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. In particular, p38 MAPKα is activated by phosphorylation at Thr180 and Tyr182, which is mediated by a variety of cellular stresses including inflammatory cytokines, UV light, osmotic shock and lipopolysaccharides.

Activated p38 MAP kinase has been shown to phosphorylate and activate several downstream kinases and transcription factors implicated in regulating stress related transcription and the control of cell cycle.

## 2. Protocol Summary

Prepare all reagents, samples, and controls as instructed

Add 50 µL control or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Substrate to each well and incubate for 15 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

# 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 +4°C immediately upon receipt.**

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.

## 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 | Quantity | Storage Condition |
|---|---|---|
| p38 MAPKα (pT180/Y182) Capture Antibody | 3mL | +4°C |
| p38 MAPKα (pT180/Y182) Detector Antibody | 3mL | +4°C |
| Control Lysate | 1 Vial | +4°C |
| 10X Wash Buffer PT | 15 mL | +4°C |
| 5X Cell Extraction Buffer PTR | 12 mL | +4°C |
| 50X Cell Extraction Enhancer Solution | 1 mL | +4°C |
| TMB Substrate | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| Pre-Coated 96 Well Microplate (12 x 8 well strips) | 96 Wells | +4°C |
| Plate Seal | 1 | +4°C |

**Control Lysate:**
Prepared from HeLa cells, cultured to confluence in T175 flasks in 10% FBS containing medium, then treated with 2µg/mL anisomycin for 15min.

## 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 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for Control lysate dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
- PBS.

## 8. Technical Hints

- Samples generating values higher than the highest Control Lysate 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, control and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided 5X Cell Extraction Buffer contains phosphatase inhibitors. Protease inhibitors can be added if required.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or controls to the well before the addition of the Antibody Cocktail.
- 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 or control 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 to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The reagent volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 2X formulations.

## 9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

## 9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 60 mL 1X Wash Buffer PT combine 6 mL 10X Wash Buffer PT with 54 mL deionized water. Mix thoroughly and gently.

## 9.3 5X Cell Extraction/Enhancer Buffer PTR:

Prepare 5X Cell Extraction/Enhancer Buffer PTR by adding 1/10th volume of 50X Cell Extraction Enhancer Solution. To prepare 1 mL, add 100 µL of 50X Cell Extraction Enhancer Solution to 900 µL of 5X Cell Extraction Buffer PTR. This concentrated mix is used for lysing cells directly in cell culture medium.

## 9.4 Antibody Cocktail:

Prepare Antibody Cocktail by combining an appropriate volume of the capture and detector antibodies immediately prior to assay. To make 3 mL of the Antibody Cocktail combine 1.5 mL Capture Antibody with 1.5 mL Detector Antibody. Mix thoroughly and gently.

## 10. Control Lysate Preparation

Kit Control lysates are provided at a concentration that give consistent signal between different lots. Lysates are produced and formulated by signal intensity to be consistent to within 30% of the previous lot. As such, Control lysates are not provided with a protein concentration.

- Always prepare a fresh set of positive controls for every use.
- Prepare serially diluted control lysates immediately prior to use.
- Discard working lysate dilutions after use as they do not store well.
- The following section describes the preparation of a lysate dilution series for duplicate measurements (recommended).

**10.1** Reconstitute the Lyophilized p38 MAPK Control Lysate* by adding 250 µL water. Mix thoroughly and gently. Hold at room temperature for 1 minute and mix gently. This is the 100% **Stock Lysate** Solution. Remaining stock material should be aliquoted and stored at -80 °C.

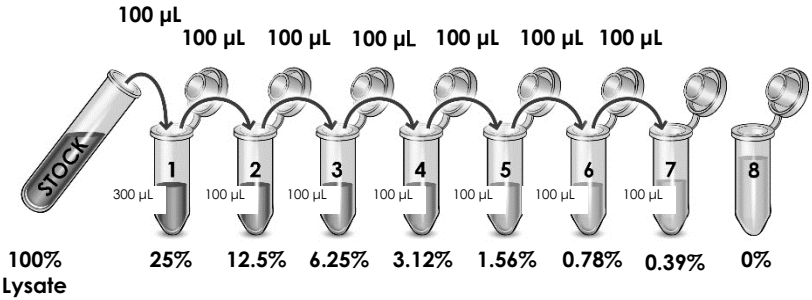
**10.2** Label eight tubes, Controls 1– 8.

**10.3** Add 300 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 100 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.

**10.4** Use the Stock Lysate to prepare the following dilution series. Control #8 contains no protein and is the Blank control:

*Control lysates are supplied as a control reagent - not an absolute quantitation measure. A 3 - 4 point lysate dilution series is sufficient for this purpose.

Note: the extent of the dilution series appropriate for your samples needs to be determined empirically. Further dilutions than those shown below may be required.



# 11. Sample Preparation

- A cell density that yields 10,000 – 40,000 cells/well is suitable for the analysis of many cell lines. The lysis buffer volume should be adjusted so that lysates are in the range of 100-500 µg/mL of protein.

## 11.1 Preparation of extracts from cell pellets:

11.1.1 Collect non-adherent cells by centrifugation and resuspend at an appropriate density in RPMI containing 10% FBS. Typical centrifugation conditions for cells are 500 x g for 5 minutes at RT.

11.1.2 Return cells to a 37°C incubator for 1 - 2 hours. For certain pathways, this can allow handling-mediated pathway activation to subside. This step is optional, and depends on the activation status of your cells following re-suspension.

11.1.3 At the completion of the cell treatment, harvest cells by centrifugation and lyse with 1X Cell Extraction Buffer PTR.*

11.1.4 Alternatively, in the absence of centrifugation cells may be lysed directly with a 20% volume of 5X Cell Extraction/Enhancer Buffer PTR (e.g. for 80 µL of cells, use 20 µL of 5X Cell Extraction/Enhancer Buffer PTR).

11.1.5 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.6 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

*For best results, we recommend centrifugation and lysis of cells with 1X Cell Extraction Buffer PTR (11.2.3). Matrix effects may be observed in different cell media using the direct lysis approach (11.2).

## 11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (for cells cultured in 96-well microplates, lyse the cells with 100 µL* of 1X Cell Extraction Buffer PTR).

*Lysis volume should be adjusted depending on the desired lysate concentration. Lysates in the range of 100 - 500 µg/mL protein are usually sufficient.

11.2.3 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.4 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## 11.3 Preparation of extracts from tissue homogenates:

11.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes.

11.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## 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 plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

## 13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

13.1 Prepare all reagents, working controls, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or control to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Substrate begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
|---|---|
| Wavelength: | 600 nm |
| Time: | up to 15 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

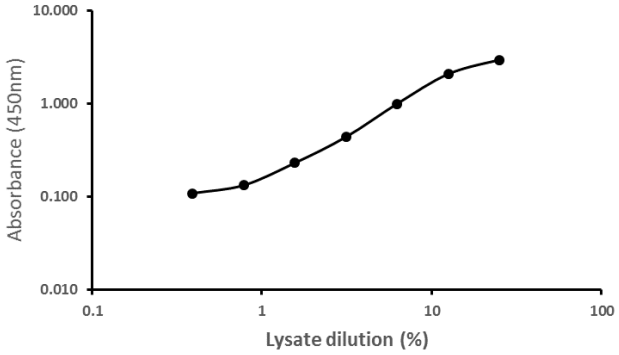
13.10 Analyze the data as described below.

# 14. Calculations

*Control Lysates are supplied as a control reagent – not an absolute quantitation method.

# 15. Typical Data

**Typical cell lysate dilution series** – data provided **for demonstration purposes only**. A new lysate dilution series must be generated for each assay performed.

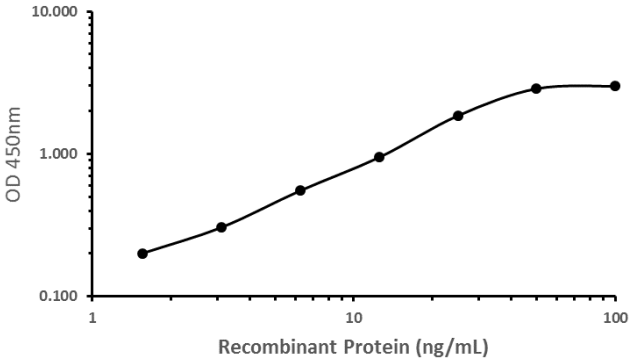



| Lysate Dilution Series Measurements | | | |
|---|---|---|---|
| Control Lysate (%) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0.000 | 0.075 | 0.071 | 0.073 |
| 0.39 | 0.107 | 0.110 | 0.109 |
| 0.78 | 0.126 | 0.140 | 0.133 |
| 1.56 | 0.235 | 0.227 | 0.231 |
| 3.12 | 0.438 | 0.439 | 0.439 |
| 6.25 | 1.043 | 0.955 | 0.999 |
| 12.5 | 2.085 | 2.075 | 2.080 |
| 25 | 2.947 | 2.943 | 2.945 |

**Figure 1**. Example of a typical p38 MAPKα (pT180/Y182) control lysate dilution series in 1X Cell Extraction Buffer PTR. The p38 MAPKα lysate dilution series was prepared as described in Section 10. Raw data values are shown in the table.

Kit Control lysates are provided at a concentration that give consistent signal between different lots. Lysates are produced and formulated by signal intensity to be consistent to within 30% of the previous lot. As such, Control lysates are not provided with a protein concentration.

**Typical recombinant protein standard curve –** data provided **for demonstration purposes only.**




| Recombinant Protein Curve Measurements | | | |
|---|---|---|---|
| Concentration (ng/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0.000 | 0.052 | 0.054 | 0.053 |
| 1.56 | 0.203 | 0.199 | 0.201 |
| 3.12 | 0.303 | 0.308 | 0.306 |
| 6.25 | 0.556 | 0.553 | 0.555 |
| 12.50 | 0.957 | 0.942 | 0.950 |
| 25.00 | 1.877 | 1.853 | 1.865 |
| 50.00 | 2.874 | 2.903 | 2.889 |
| 100.0 | 2.951 | 3.049 | 3.000 |

**Figure 2**. Example of a typical p38 MAPKα (pT180/Y182) recombinant protein standard curve. The proportion of total protein that is phosphorylated is unknown - data is indicative only. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

# 16. Typical Sample Values

## SENSITIVITY –

Important: It should be noted that absolute mass quantitation of the phosphorylated target is not possible. The graph and values above (Section 15) are of an indicative nature only. The proportion of the total target protein that is phosphorylated in either a cell control lysate or a recombinant protein sample are unknown, and therefore cannot be used to interpolate to actual levels of phosphorylated target in a biological test sample. However, levels of target relative to, for example, unstimulated levels in the same experimental preparation, can be accurately determined.

As a guide, p38 MAPKα (pT180/Y182) is detectable in activated HeLa extracts (anisomycin treated) with a total cellular protein concentration < 5 µg/mL (see Figure 1).

## PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of HeLa extracts within the working range of the assay.

| Sample Type | Intra Assay | Inter Assay |
|---|---|---|
| n= | 6 | 3 |
| CV (%) | 2.3 | 8.3 |

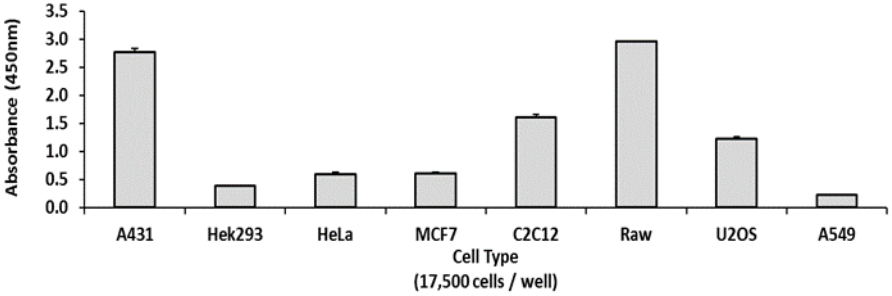



**Figure 3.** Cell line analysis for p38 MAPKα Total from preparations of 17,500 cells/well cell extracts. Data from triplicate measurements (mean +/- SD) are plotted.

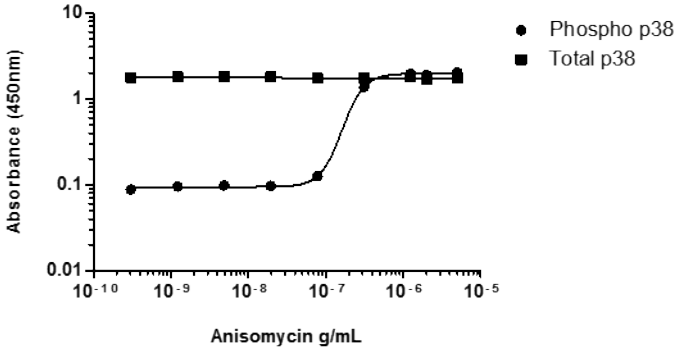



**Figure 4.** Induction of p38 MAPKα (pT180/Y182) phosphorylation in HeLa cells in response to anisomycin treatment, and levels of p38 MAPKα Total in the same samples. HeLa cells were cultured in 96-well tissue culture plates, serum starved (2 hr), and treated (30 min) with a dose-range of anisomycin before cell lysis. Data from duplicate measurements of p38 MAPKα (pT180/Y182) are plotted and compared against p38 MAPKα Total protein levels.

## 17. Assay Specificity

The p38 MAPKα (pT180/Y182) assay detects endogenous levels of p38 MAPKα (GenBank Accession NP_001306) in cellular lysates, only when phosphorylated at Thr180/Tyr182.

## 18. Species Reactivity

This kit detects p38 MAPKα (pT180/Y182) in Human and mouse cell culture extracts. Detection in rat samples is also expected. Other species should be tested on a case-by-case basis.

Serum and plasma samples have not been tested with this kit.

Please contact our Technical Support team for more information.

# 19. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| **Difficulty pipetting lysate; viscous lysate.** | Genomic DNA solubilized | Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction. |
| **Poor Control Lysate series curve** | Inaccurate Pipetting | Check pipettes |
| | Improper Lysate dilution | Prior to opening, briefly spin the stock Control Lysate tube and dissolve the powder thoroughly by gentle mixing |
| **Low Signal** | Incubation times too brief | Ensure sufficient incubation times; increase to 2 or 3 hour lysate/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Incubation times with TMB too brief | Ensure sufficient incubation time until blue color develops prior addition of Stop solution |
| **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 ELISA kit | Store your reconstituted Control Lysate at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light. |
| **Precipitate in 50X Cell Extraction Enhancer Solution** | Precipitation and/or coagulation of components within the 50X Cell Extraction Enhancer Solution. | Precipitate can be removed by gently warming the 50X Cell Extraction Enhancer Solution to 37°C. |

# 20. 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)