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ab219177
Nuclear Extract Kit

For the preparation of nuclear extracts from mammalian cells and tissue.

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

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1. Overview

ab219177 provides a simple method for extracting nuclear proteins from mammalian cells or tissues in 45 minutes. For optimal extraction of nuclear proteins, the kit produces three distinct extracts (fractions): soluble nuclear proteins (nuclear extract 1), nuclear proteins that remain insoluble after production of nuclear extract 1 (nuclear extract 2), and cytoplasmic proteins. The kit contains reagents for 100 extractions, where each extraction starts with 5 x 10^6 cells or 50 mg of tissue. Typical yields from each extraction are 0.3 – 0.4 mg of soluble nuclear proteins, 0.1 – 0.2 mg of insoluble nuclear proteins and 0.6 – 0.7 mg of cytoplasmic proteins. Nuclear proteins extracted with this kit can be used in a variety of applications such as western blotting and nuclear enzyme assays.

2. Protocol Summary

Prepare cell suspension / homogenize & disrupt tissue

Extract cytoplasmic proteins

Extract nuclear proteins (Nuclear Extract 1)

Extract nuclear proteins that were insoluble in previous step (Nuclear Extract 2)

Quantify proteins for use in desired application

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. 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.

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 temperature
200X Protease Inhibitor Cocktail	1 mL	-20°C
200X DTT	1 mL	-20°C
Cytoplasm Extraction Buffer	55 mL	4°C
Nuclear Extraction Buffer	55 mL	4°C
Nuclear Lysis Buffer	55 mL	4°C

7. Materials Required, Not Supplied

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

- 1X Phosphate-buffered saline PBS (e.g. ab64026, ab64246)
- Trypsin/EDTA solution
- Double-distilled water (ddH2O)
- 1.5 mL and 15 mL plastic tubes
- Benchtop microcentrifuge
- Centrifuge for 15 mL tubes
- Sonicator

8. Technical Hints

- **This kit is sold based on number of tests. One test corresponds to the fractionation of 5 x 10^6 cells. 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 and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- 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 Protease Inhibitor Cocktail:

On receipt of the kit, aliquot and store at -20°C. Ready to use as supplied. Thaw aliquot before use. Avoid repeated freeze-thaw cycles.

9.2 200X DTT:

On receipt of the kit, aliquot and store at -20°C. Ready to use as supplied. Thaw aliquot before use. Avoid repeated freeze-thaw cycles.

9.3 Cytoplasm Extraction Buffer:

Ready to use as supplied. Store at 4°C.

9.4 Nuclear Extraction Buffer:

Ready to use as supplied. Store at 4°C.

9.5 Nuclear Lysis Buffer:

Ready to use as supplied. Store at 4°C.

10. Assay Procedure

- Ensure that PBS is at 4°C before starting the experiment and store on ice until required. If starting with adherent cells, keep some PBS at room temperature for washing the cells while they are still adhered to the plate.
- Cool the benchtop microcentrifuge to 4°C before starting the experiment.
- If the extracts prepared will be used in enzyme activity assays or other downstream applications that are influenced by protease inhibitors, do not add Protease Inhibitor Cocktail to any buffers or fractions.

10.1 Buffer preparation:

10.1.1 For each extraction of 5 x 10^6 cells or 50 mg of tissue, transfer 500 µL of Cytoplasmic Extraction Buffer, 500 µL of Nuclear Extraction Buffer and 500 µL of Nuclear Lysis Buffer into clean 1.5 mL microcentrifuge tubes and store on ice.

10.1.2 To each tube, add 2.5 µL of 200X Protease Inhibitor Cocktail and 2.5 µL of 200X DTT. Keep tubes on ice until required.

Δ Note: do not use Protease Inhibitor Cocktail if the nuclear extract produced using this kit will be used for enzyme activity assays or other downstream applications that may be affected by the presence of protease inhibitors.

10.1.3 Chill PBS to 4°C and store on ice.

10.2 Adherent cells:

10.2.1 Grow cells to 70-80% confluency on a culture plate or flask (about 2-5 x 10^6 cells for a 100 mm plate). Remove the growth medium. Wash the cells with room temperature PBS twice, then remove PBS.

10.2.2 Add 1 mL of room temperature PBS per 20 cm^2 areas of cells (e.g. add 3 mL of PBS to a 100 mm plate) and scrape cells into a 15 mL tube. Alternatively, dispense enough trypsin/EDTA solution to completely cover the monolayer of cells and incubate in a 37°C incubator for about 2 minutes or until cells detach from the surface. When the trypsinization process is complete, the cells will be in suspension and appear rounded. Add serum or media containing serum to the cell suspension as soon as possible to protect cells from damage caused by tryptic activity.

Δ **Note:** trypsinization may have an impact on the cellular pathway of interest.

10.2.3 Count cells using a hemacytometer.

10.2.4 Centrifuge the cells for 5 minutes at 1000 rpm at 4°C and discard the supernatant.

10.2.5 Wash cells with 10 mL of ice-cold PBS by centrifugation for 5 minutes at 1000 rpm at 4°C. Discard the supernatant.

10.2.6 Proceed to step 10.5.

10.3 Suspension cells:

10.3.1 Grow cells to 2 x 10^6 / mL and collect the cells in a 15 mL tube.

10.3.2 Centrifuge the cells for 5 minutes at 1000 rpm at 4°C and discard the supernatant.

10.3.3 Wash cells with PBS by centrifugation for 5 minutes at 1000 rpm at 4°C. Discard the supernatant.

10.3.4 Proceed to Step 10.5.

10.4 Tissues:

10.4.1 Weigh the tissue and cut it into small pieces to facilitate homogenization.

10.4.2 Wash tissue twice with ice-cold PBS.

10.4.3 Transfer tissue into a clean, chilled Dounce homogenizer and add 500 µL of ice-cold Cytoplasm Extraction Buffer supplemented with protease inhibitors and DTT. Keep sample on ice.

10.4.4 Disrupt tissue with 30 strokes.

Δ **Note:** View the sample under a microscope to check that the tissue has been homogenized and that most cells have been released into solution. If required, apply more strokes to complete homogenization. Alternatively, pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization – however, please consider whether this procedure may result in sample misrepresentation.

10.4.5 Transfer homogenized tissue into fresh tube and incubate on ice for 10 minutes.

10.4.6 Vortex briefly.

10.4.7 Proceed to Step 10.5.4.

10.5 Extraction of cytoplasm:

10.5.1 Resuspend cell pellet in 500 µL of ice-cold Cytoplasm Extraction Buffer with protease inhibitors and DTT by gently pipetting up and down and transfer to a 1.5 mL tube.

10.5.2 Vortex briefly.
10.5.3 Incubate cells on ice for 10 minutes.
10.5.4 Vortex briefly and centrifuge suspension for 3 min at 1000 g in a pre-cooled bench-top microcentrifuge.
10.5.5 Carefully remove as much supernatant as possible and keep both the pellet and the supernatant on ice. The supernatant is the cytoplasmic protein extract and may be quantified and used for downstream applications. The pellet is usually viscous and not very compact.

10.6 Extraction of soluble nuclear proteins:

10.6.1 Resuspend pellet from Step 10.5.5 in 500 µL of ice-cold Nuclear Extraction Buffer with protease inhibitors and DTT.
10.6.2 Vortex briefly.
10.6.3 Incubate cells on ice for 15 minutes. Vortex every 5 minutes during the incubation.
10.6.4 Vortex briefly.
10.6.5 Centrifuge for 3 minutes at 5000 g in a benchtop microcentrifuge at 4°C.
10.6.6 Carefully remove as much supernatant as possible and store both the supernatant and the pellet on ice. The supernatant is enriched for soluble nuclear proteins (Nuclear Extract 1).

10.7 Extraction of insoluble nuclear proteins:

10.7.1 Resuspend pellet from Step 10.6.6 in 500 µL mL of ice-cold Nuclear Lysis Buffer with protease inhibitors and DTT.
10.7.2 Briefly sonicate the sample on ice until the sample is homogenous, the nuclei have been lysed and the genomic DNA has been sheared. This fraction (Nuclear Extract 2) contains all remaining nuclear proteins that were not extracted in previous steps, e.g. histones. Keep sample on ice.

Δ Note: The amount of sonication required will depend on a number of factors such as cell type. Use a microscope to verify that the nuclei are lysed. If pipetting is problematic (due to release of genomic DNA), treat sample with a few more bursts of ultrasound. Always use as little sonication as possible to homogenize the sample, ensure that the sample is cold and keep the sonication protocol consistent between samples.

10.8 Protein quantification and analysis:

10.8.1 Measure the protein concentration of the fraction(s) of interest. We recommend using BCA protein assay kit reducing agent compatible ab207003 or ab207004, as these assays have been developed for accurate protein quantification in the presence of reducing agents.

10.8.2 Use the fraction(s) of interest immediately or aliquot and freeze at -80°C.

Δ Note: If analyzing the fractions by Western blot, ensure that EDTA is present in the sample to a final concentration of at least 5 mM. EDTA may be added to the sample before denaturation or to the sample buffer (always check your sample buffer first as some recipes contain EDTA by default).

11. Typical data

Data provided for **demonstration purposes** only.

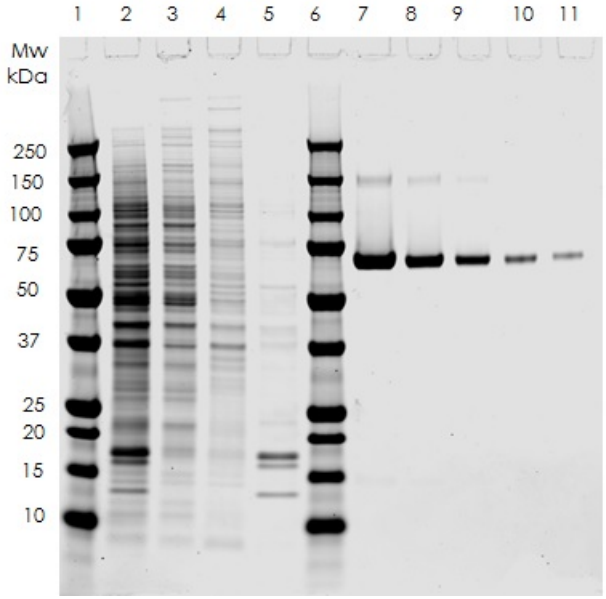

Figure 1. SDS PAGE analysis of HeLa cell fractionation using Nuclear Extract Kit ab219177. Extracts were prepared from $5x10^6$ HeLa cells using Nuclear Extract Kit ab219177 and analysed by SDS-PAGE with Coomasie Blue staining. Extract or whole cell lysate from $3x10^4$ cells was loaded in each lane along with BSA standards. Lanes: 1. MW marker; 2. Whole cell lysate; 3. Cytoplasmic extract; 4. Nuclear extract 1; 5. Nuclear extract 2; 6. MW marker; 7. BSA, 2.0 µg; 8. BSA 1.0 µg; 9. BSA 0.5 µg; 10. BSA 0.2 µg; 11. BSA 0.1 µg.

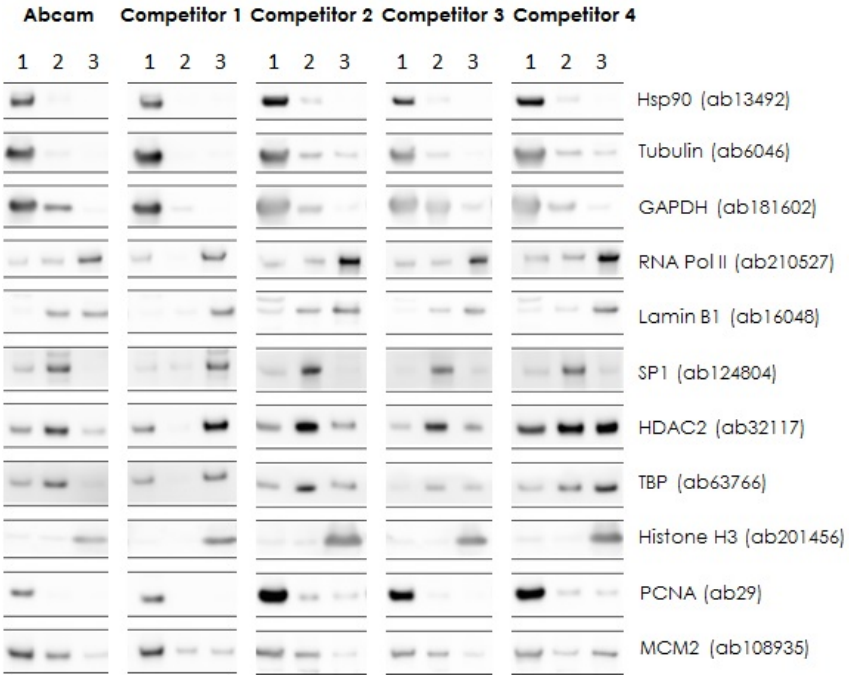

Figure 2. Comparison of HeLa cell fractionation by Nuclear Extract Kit ab219177 and leading commercially available nuclear fractionation kits. Extracts were prepared from HeLa cells using the manufacturer's protocol and analyzed by Western blot. For each manufacturer: (1) cytoplasmic extract, (2) nuclear extract, (3) nuclear extract 2 or resolubilized insoluble nuclear fraction.

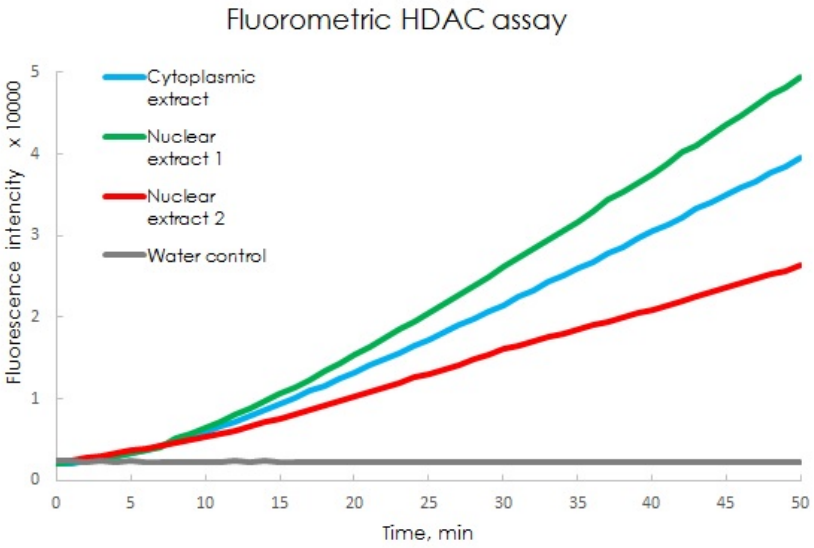

Figure 3. Measurement of Histone Deacetylase (HDAC) activity in HeLa cell fractions prepared using Nuclear Extract Kit ab219177. Extracts were prepared from $5x10^6$ HeLa cells using Nuclear Extract Kit ab219177. Note that protease inhibitors were omitted from all buffers to prevent interference with the HDAC activity assay. The enzymatic activity of HDACs in each fraction was measured using the Histone Deacetylase (HDAC) Activity Assay Kit (Fluorometric) (ab156064). Extract from of $3x10^4$ cells was used per assay test.

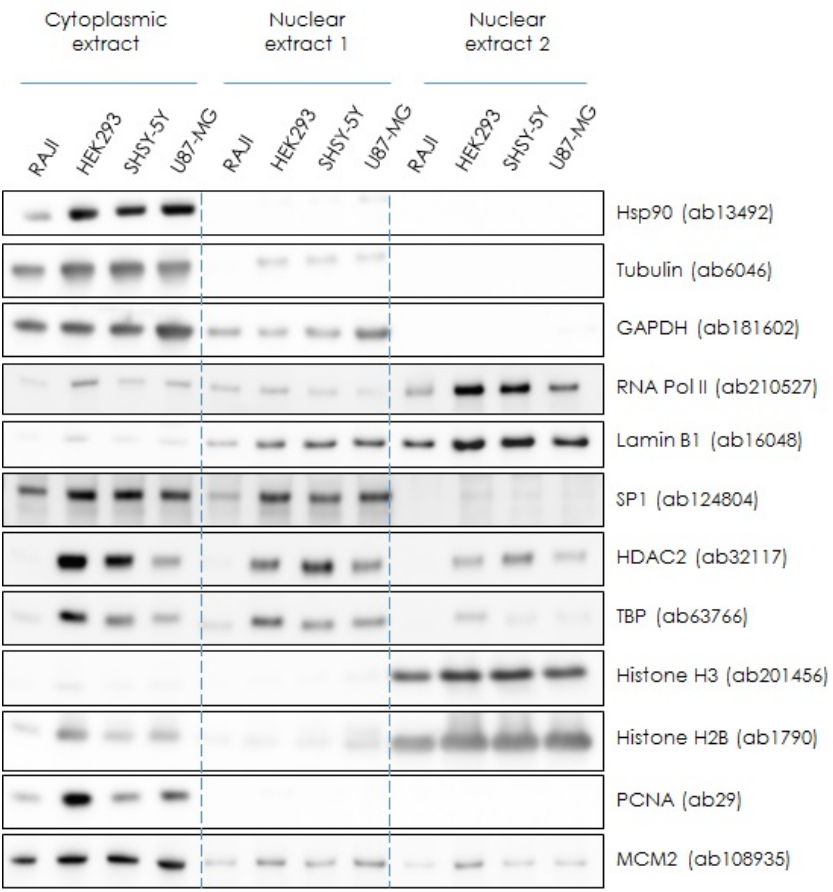

Figure 4. Comparison of nuclear extraction from different cell lines. Extracts were prepared from $5x10^6$ cells from RAJI (Human lymphoblastoid cell line), HEK293 (Human embryonic kidney cell line), SHSY-5Y (Human neuroblastoma cell line) and U87-MG (Human glioblastoma cell line) cell lines using Nuclear Extract Kit ab219177 and analysed by Western blot. Extract from $3x10^4$ cells was loaded per lane.

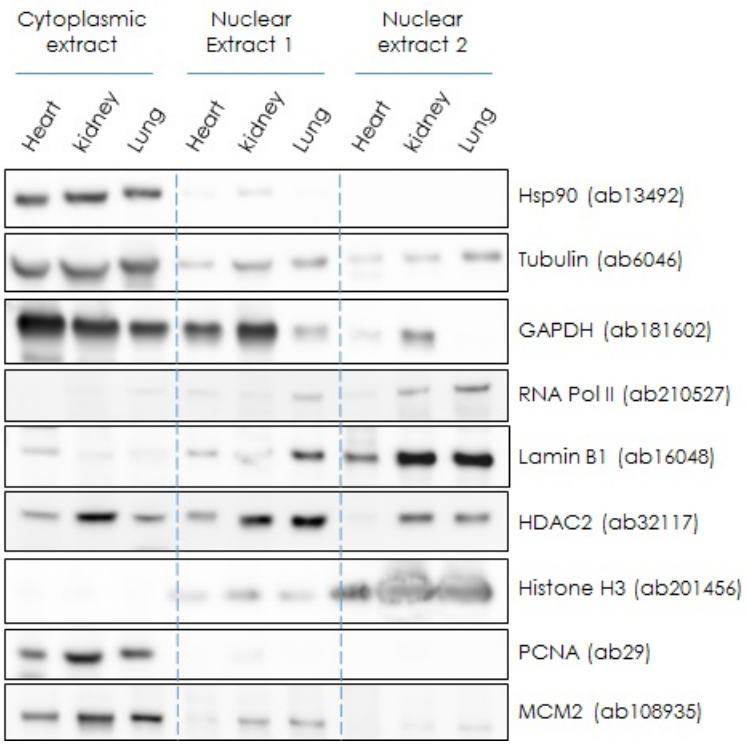

Figure 5. Comparison of nuclear extraction from different tissues. Extracts were prepared from120 mg of mouse tissue using Nuclear Extract Kit ab219177 and analysed by Western blot. Extract from 0.4 mg of tissue was loaded per lane.

12. Troubleshooting

Problem	Reason	Solution
Low yield of nuclear proteins.	Insufficient starting material.	To obtain the best results, the starting material should be $5x10^6$ cells, or 50 mg of tissue. Note that yields will vary depending on cell or tissue type so the amount of starting material may have to be optimized.
	Tissue samples were not homogenized sufficiently.	Increase the number of strokes with the Dounce homogenizer or explore alternative methods/instruments for tissue homogenization.
		Following homogenization, pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization. Note that this may result in sample misrepresentation.
		Check, using a microscope, that cells are well resuspended and that clump/aggregates are minimal.
	Cell pellets were not resuspended thoroughly.	Increase number of times the pellet is pipetted up and down. Use a microscope to validate that cells are well resuspended and that clump/aggregates are minimal.
	Incomplete lysis of nucleus.	Sonicate the sample to increase the yield of nuclear extracts. If the sonication step was performed, increase the sonication time or number of cycles until complete lysis is achieved. Use a microscope to confirm lysis.
	Reagents have expired. Expired reagents may cause inefficient extraction.	Ensure that the kit has not exceeded the expiration date.
	Incorrect temperature and/or	Ensure the incubation time and temperatures described in the

	insufficient incubation time during extraction.	protocol are followed.
Low/no activity of nuclear enzymes in downstream activity assays.	Improper starting material.	The enzymatic activity of nuclear extracts from frozen cells or tissue may be much lower than that from fresh tissues and therefore using fresh cells or tissue is recommended whenever possible. Nuclear extracts should be stored at -80°C (3-6 months). Avoid multiple freeze/thaw cycles.
	Protease inhibitors are incompatible with downstream enzymatic activity assay.	Protease inhibitors can be omitted from the fractionation procedure but the sample integrity may be compromised – keep samples on ice to minimize proteolytic degradation.
	Enzyme of interest has degraded.	Maintain low temperatures throughout procedure to minimize degradation. Minimize procedure time as much as possible. Use extract in assay immediately after preparation. Add protease inhibitors that do not affect the activity of the enzyme of interest.
When performing protein concentration measurement: 1) the blank and samples are all saturated; 2) the blank and samples turned a dark purple colour immediately upon adding nuclear extracts or final working buffer for blank.	The protein quantification assay is not compatible with DTT present in the final working buffer.	1) Use a protein quantification assay that is compatible with DTT, e.g.BCA protein assay kit reducing agent compatible ab207003 or ab207004. 2) Measure nuclear protein concentration before adding DTT. After the protein concentration has been measured the DTT can be added for storage purposes.

13. 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)