ab189569 – IL-11 (Interleukin-11) Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of IL-11 (Interleukin-11) in human serum, plasma and cell culture supernatant samples.

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

Version 7a, Last Updated 27 May 2025

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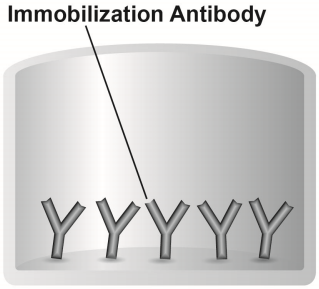
1. BACKGROUND

IL-11 (Interleukin-11) in *vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-11 protein in human serum, plasma and cell culture supernatant samples.

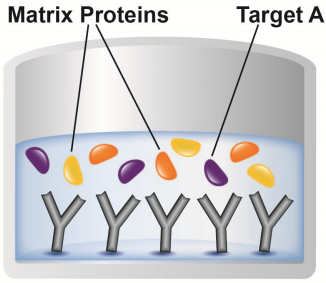
The SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. 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.

IL-11 is multifunctional cytokine of the IL-6 superfamily that is secreted by many cell types and is best known for its hematopoietic activity. IL-11 directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces megakaryocyte maturation resulting in increased platelet production. During embryonic development L-11 signaling is required for normal development of placentation and survival to birth.

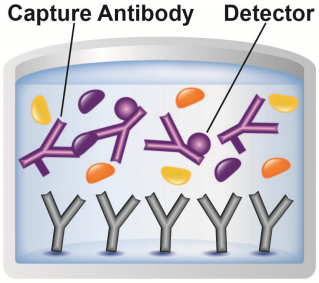
2. ASSAY SUMMARY



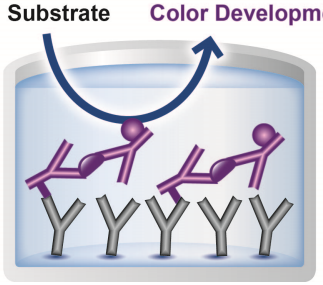
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

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 at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X IL-11 Capture Antibody	600 µL	+2-8ºC
10X IL-11 Detector Antibody	600 µL	+2-8ºC
IL-11 Human Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent 5BI	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Sample Diluent 50BS	20 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm
- Method for determining protein concentration (BCA assay recommended)
- Deionized water
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4)
- Multi- and single-channel pipettes
- Tubes for standard dilution
- Plate shaker for all incubation steps
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors)

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

- Samples generating values higher than the highest standard 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, standard 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
- **To avoid high background always add samples or standards 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, 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 to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample 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 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

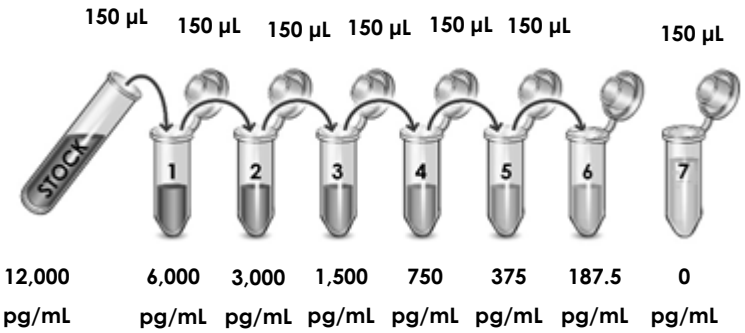
10.1 For measurements of **cell culture supernatant** samples:

10.1.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the IL-11 standard by adding that volume of water indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IL-11 standard by adding 200 µL water. Hold at room temperature for 10 minutes and mix gently. This is the 12,000 pg/mL **Stock Standard Solution**.

10.1.2 Label seven tubes, Standards 1 – 7.

10.1.3 Add 150 µL Sample Diluent NS into tube numbers 1-7.

10.1.4 Use the Stock Standard to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:



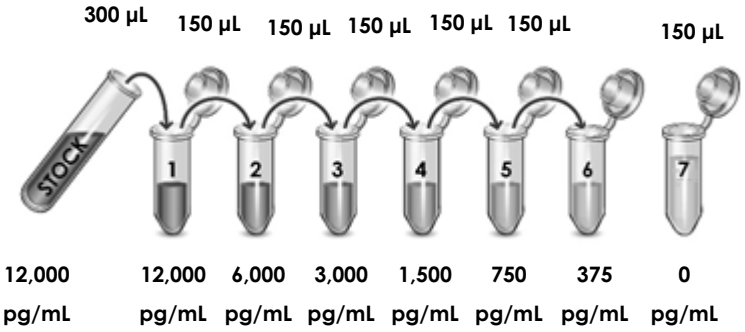
10.2 For **measurements of serum and plasma (EDTA) samples**:

10.2.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the IL-11 standard by adding that volume of water indicated on the label. Reconstitute the IL-11 Human Lyophilized Recombinant Protein standard sample by adding 200 µL Sample Diluent 50BS by pipette. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 12,000 pg/mL **Stock Standard** Solution.

10.2.2 Label eight tubes, Standards 1 – 7.

10.2.3 Add 150 µL Sample Diluent 50BS into tube numbers 2-7.

10.2.4 Use the Stock Standard to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
PMA + IL-1α- treated MRC5 supernatant	8X– 64X diluted

11.1 Plasma

Collect plasma using EDTA. Centrifuge samples at 2,000 x g for 10 minutes and collect plasma. Neat plasma samples can be assayed without dilution. If needed, dilute plasma samples into Sample Diluent 50BS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles. **This kit is not compatible with citrate- and heparin-plasma samples.**

11.2 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Neat serum samples can be assayed without dilution. If needed, dilute serum samples into Sample Diluent 50BS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris and collect supernatants. Dilute cell culture supernatant samples at least 4 X into Sample Diluent NS and assay. Store undiluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, 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 4ºC storage.

13.3 Add 50 µL of all sample or standard 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. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10-15 minutes in the dark (avoid standard signal saturation) on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

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.

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 Development Solution 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 20 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.

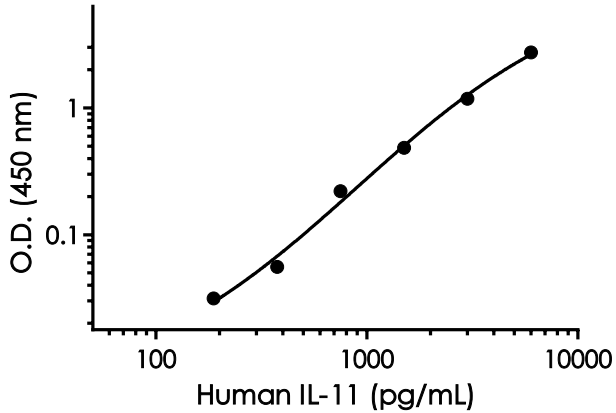
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually 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.

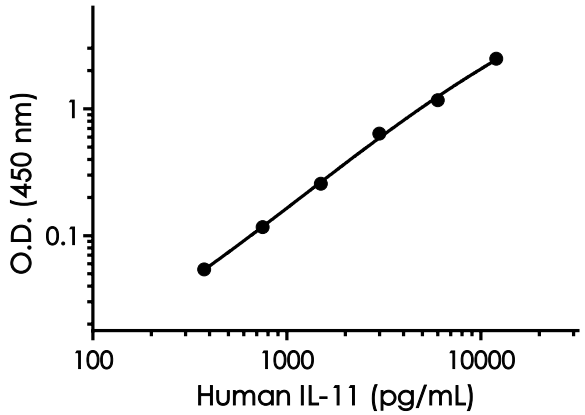
15. TYPICAL DATA

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



Standard Curve Measurements			
Conc. (pg/mL)	**O.D. 450 nm**		**Mean O.D.**
	1	**2**	
0	0.100	0.115	0.108
187.5	0.150	0.130	0.140
375	0.187	0.146	0.167
750	0.327	0.327	0.327
1,500	0.584	0.598	0.591
3,000	1.257	1.316	1.287
6,000	2.949	2.754	2.852

Figure 1. Example of IL-11 standard curve for cell culture supernatant samples measurements. The IL-11 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.053	0.048	0.051
375	0.104	0.104	0.104
750	0.169	0.165	0.167
1,500	0.321	0.292	0.307
3,000	0.698	0.675	0.687
6,000	1.204	1.232	1.218
12,000	2.420	2.651	2.536

Figure 2. Example of IL-11 standard curve for serum and plasma (EDTA) samples measurements. The IL-11 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations:

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	8	169.8 pg/mL
Sample Diluent 50BS	8	83.3 pg/mL

RECOVERY –

Three concentrations of Human recombinant IL-11 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
100% Human Serum	99.6	94.9 – 104.4
100% Human Plasma - EDTA	116.9	108.3 – 125.3
25% 10F HGDMEM media	98.5	93.5 – 104.3

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native IL-11 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	12.5% MRC5-(PMA+IL-1α) SN
Undiluted	pg/mL	1,714.0
	% Expected value	**100.0**
2	pg/mL	757.4
	% Expected value	**88.4**
4	pg/mL	405.8
	% Expected value	**94.7**
8	pg/mL	219.7
	% Expected value	**102.6**

Recombinant Human IL-11 was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 50BS.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (EDTA)
Undiluted	pg/mL	3,463.1	4,150.2
	% Expected value	**100.0**	**100.0**
2	pg/mL	1,586.9	1,674.9
	% Expected value	**91.6**	**80.7**
4	pg/mL	848.5	874.8
	% Expected value	**98.0**	**84.3**
8	pg/mL	482.0	474.7
	% Expected value	**111.3**	**91.5**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of PMA + IL-1α - treated MRC5 supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	5	3
CV (%)	1.7	10.3

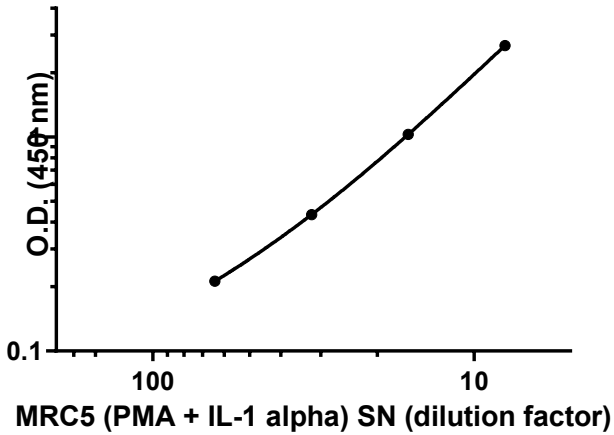


Figure 3. Titration of PMA + IL-1 alpha - treated MRC5 supernatant within the working range of the assay. MRC5 cells were grown in 10F HGDMEM media in the presence of 10 ng/mL phorbol 12-myristate 13-acetate (PMA) and 1 ng/mL IL-1 alpha (ab9616) for 48 hours. IL-11 concentrations were measured in serial dilutions of cell culture supernatants using this kit. Background-subtracted data values (mean +/- SD, n = 2) are graphed.

Serum/Plasma. Neat pooled Human serum and plasma (EDTA) samples (each pooled from 50 apparently healthy donors), as well as neat individual male Human donor sera were measured in triplicates for IL-11 using this kit. No detectable IL-11 concentrations in any of the above samples were observed.

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant Human IL-11 protein in serum, plasma (EDTA) and cell culture supernatant samples only.

This kit is not compatible with citrate- and heparin-plasma samples.

Cell and tissue extract samples have not been tested with this kit.

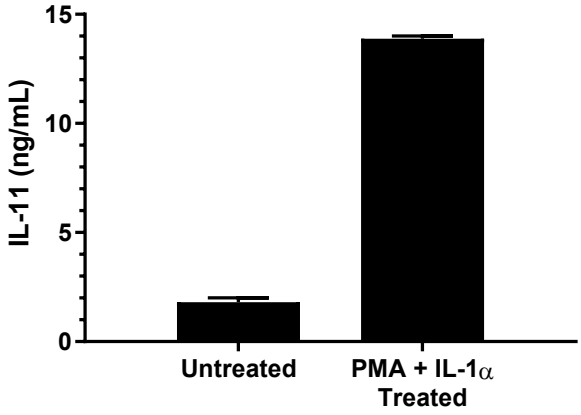


Figure 4. Comparison of IL-11 concentrations in untreated and PMA + IL-1 alpha - treated MRC5 supernatants. MRC5 cells were grown in 10F HGDMEM media in the absence or presence of 10 ng/mL phorbol 12-myristate 13-acetate (PMA) and 1 ng/mL IL-1 alpha (ab9616) for 48 hours. IL-11 concentrations were measured in 8 X diluted cell culture supernatants using this kit. Interpolated data values adjusted for sample dilution are graphed (mean +/- SD, n=3) in ng of IL-11 per mL of cell culture supernatant.

18. SPECIES REACTIVITY

This kit recognizes Human IL-11 protein.

Other species reactivity was not determined.

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard 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; increase to 2 or 3 hour standard/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 standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

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)