ab83377 Phosphatidylcholine Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of phosphatidylcholine levels in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4a, Last Updated 31 May 2023

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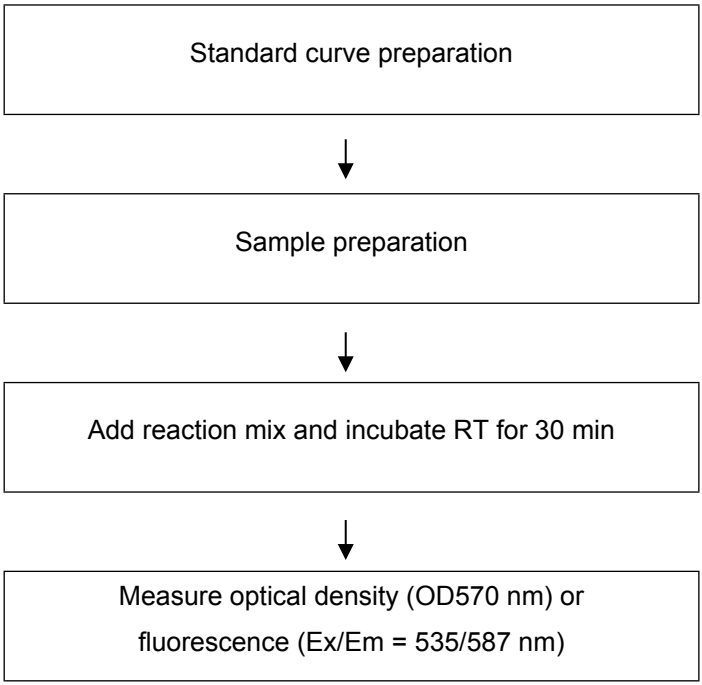
1. BACKGROUND

Phosphatidylcholine Assay Kit (colorimetric/fluorometric) (ab83377) is a simple convenient method for measuring phosphatidylcholine (PC) in a variety of biological samples. This assay uses an enzyme-coupled reaction to hydrolyze phosphatidylcholine to release choline, which subsequently oxidizes the OxiRed probe resulting in development of the Oxired Probe in order to generate fluorescence (Ex/Em 535 nm / 587 nm) or absorbance (OD=570 nm). This assay measures PC in the range of 0.1 to 10 nmol per sample.

Phosphatidylcholine (PC) is a phospholipid which incorporates choline as the headgroup of the lipid. PC is a major constituent of biological membranes and is involved in cell signalling through release of choline by phospholipase D leaving the second messenger phosphatidic acid.

PC is present in serum at ~ 0.2-2.5 mM (~50-200 mg/dL).

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 at -20ºC 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 section 5.

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

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer I/PC Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/OxiRed™ Probe	200 µL	-20°C	-20°C
PC Hydrolysis Enzyme/PC Hydrolysis Enzyme (lyophilized)	1 vial	-20°C	-20°C
Enzyme Mix IV/PC Development Mix (lyophilized)	1 vial	-20°C	-20°C
Phospholipid Standard/PC Standard (10 µmol) (lyophilized)	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

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.

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.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer I/PC Assay Buffer:**

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

9.2 **OxiRed Probe/OxiRed Probe – in DMSO:**

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 left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Keep on ice while in use.

9.3 **PC Hydrolysis Enzyme:**

Reconstitute in 220 µL Assay Buffer I/PC Assay Buffer. Pipette up and down to dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Keep on ice while in use.

9.4 **Enzyme Mix IV/PC Development Mix:**

Reconstitute in 220 µL Assay Buffer I/PC Assay Buffer. Pipette up and down to dissolve. Aliquot Enzyme Mix IV/development mix so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Keep on ice while in use.

9.5 **Phospholipid Standard/PC Standard:**

Reconstitute the Phospholipid Standard/PC Standard (10 µmol) in 200 µL of ddH_2O to generate a 50 mM Phospholipid Standard/PC standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used as soon as possible.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 500 µL of 0.5 mM Phospholipid Standard/PC standard by diluting 5 µL of the reconstituted 50mM Phospholipid Standard/PC standard with 495 µL of ddH_2O.

10.1.2 Using 0.5 mM Phospholipid Standard/PC standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.5 mM Standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	End [PC] in well
1	0	150	50	0 nmol/well
2	6	144	50	1 nmol/well
3	12	138	50	2 nmol/well
4	18	132	50	3 nmol/well
5	24	126	50	4 nmol/well
6	30	120	50	5 nmol/well

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

10.2 **For the flurometric assay:**

10.2.1 Prepare 500 µL of 0.5 mM standard by diluting 5 µL of the reconstituted 50 mM Phospholipid Standard/PC standard with 495 µL of ddH_2O.

10.2.2 Prepare 500 µL of 0.05 mM standard by diluting 50 µL of the 0.5 mM Phospholipid Standard/PC standard with 450 µL of ddH_2O.

10.2.3 Using 0.05mM Phospholipid Standard/PC standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.05 mM Standard (µL)	ddH_2O(µL)	Final volume standard in well (µL)	End [PC] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.1 nmol/well
3	12	138	50	0.2 nmol/well
4	18	132	50	0.3 nmol/well
5	24	126	50	0.4 nmol/well
6	30	120	50	0.5 nmol/well

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 cells or tissue in liquid nitrogen upon extraction and store the samples 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.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times. Incubate on ice 10 minutes.

11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 Tissue samples:

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

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes. Incubate on ice 10 minutes.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 **Plasma, Serum and Urine and other biological fluids:**

Samples can be assayed directly.

PC is present in serum at ~ 0.2-2.5 mM (~50-200 mg/dL).

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

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). ***NOTE****: for samples containing choline as it can generate significant background*.

12.2 **PC Reaction Mix (COLORIMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Background Control Reaction Mix (µL)
Assay Buffer	**44**	**46**
PC Hydrolysis Enzyme	**2**	**0**
Enzyme Mix IV/PC Development Mix	**2**	**2**
OxiRed Probe	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.3 **PC Reaction Mix (FLUOROMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Fluorometric Reaction Mix (µL)	Background Control Reaction Mix (µL)
Assay Buffer	**45.8**	**47.8**
PC Hydrolysis Enzyme	**2**	**0**
Enzyme Mix IV/PC Development Mix	**2**	**2**
OxiRe Probe*	**0.2**	**0.2**

**For fluorometric readings, using 0.2 µL/well of the probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Background Control samples + standards +1).

12.4 Add 50 µL of appropriate Reaction Mix to each standard, sample and background control sample wells.

12.5 Incubate at room temperature for 30 min protected from light.

12.6 Measure output on a microplate reader.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure 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 multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Phosphatidylcholine.

13.5 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).

13.6 Concentration of Phosphatidylcholine (as nmol/µL or mM) in the test samples is calculated as:

$$Phosphatidylcholine\ conc = \left(\frac{A}{B}\right) * D$$

Where:

A = Amount of Phosphatidylcholine in the sample well (nmol) from the standard curve.

B = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Phosphatidylcholine molecular weight: 768 g/mol.

14. TYPICAL DATA

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

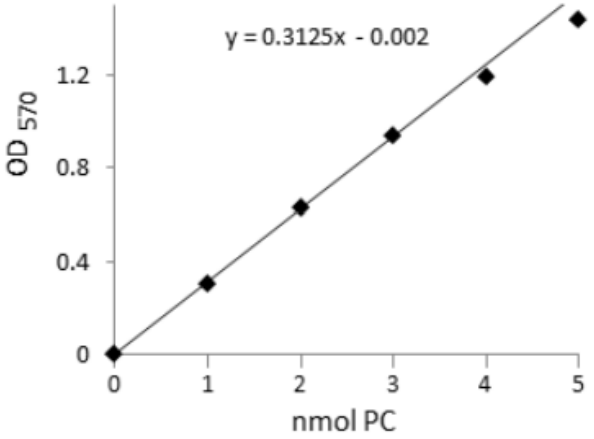


Figure 1. Typical Phosphatidylcholine standard calibration curve using colorimetric reading.

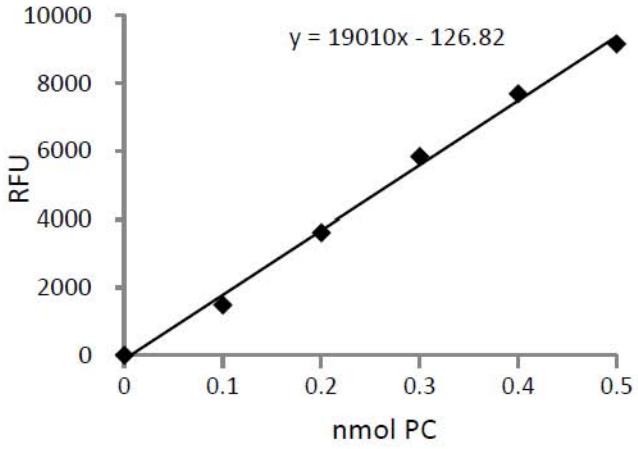


Figure 2. Typical Phosphatidylcholine standard calibration curve using fluorometric reading.

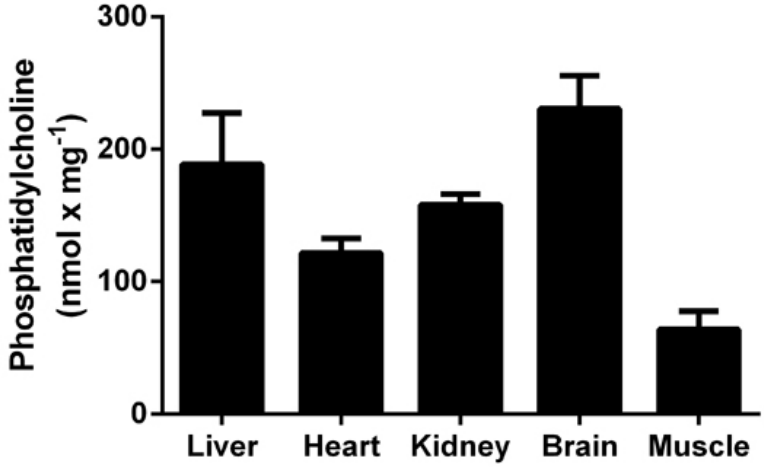


Figure 3: Phosphatidycholine levels measured fluorometrically in mouse tissue lysates.

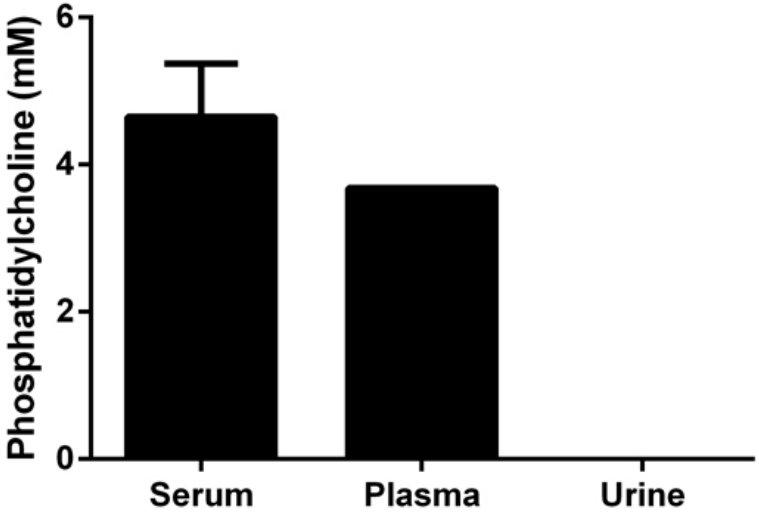


Figure 4: Phosphatidycholine levels colorimetrically measured in rat biological fluids.

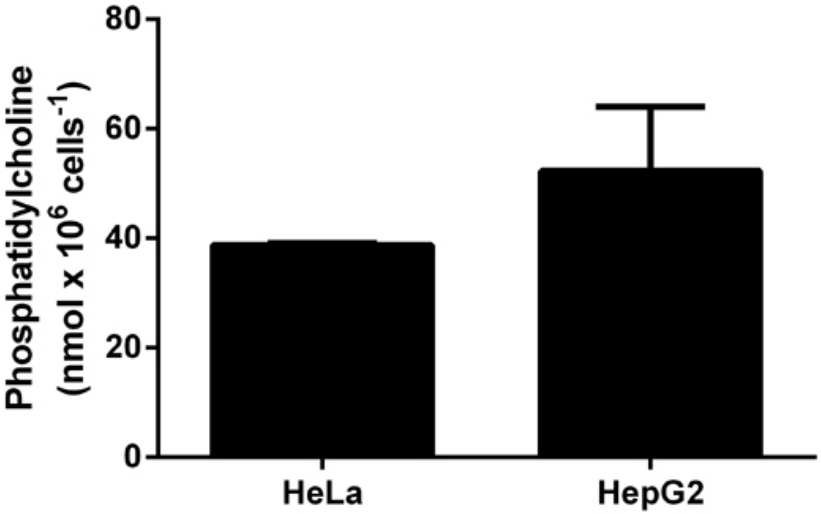


Figure 5: Phosphatidycholine levels colorimetrically measured in cell lysates.

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.*

- Prepare standard, probe, enzyme and Enzyme Mix IV/development mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings.
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare PC Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Background Control Reaction Mix (µL)
Assay Buffer	**44**	**46**
PC Hydrolysis Enzyme	**2**	**0**
Enzyme Mix IV/PC Development Mix	**2**	**2**
OxiRed Probe	**2**	**2**

Component	Fluorometric Reaction Mix (µL)	Background Control Reaction Mix (µL)
Assay Buffer	**45.8**	**47.8**
PC Hydrolysis Enzyme	**2**	**0**
Enzyme Mix IV/PC Development Mix	**2**	**2**
OxiRed Probe	**0.2**	**0.2**

- Add 50 µL of appropriate Reaction Mix to the standard, sample and background sample control wells.
- Incubate plate at RT 30 min 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 PCA precipitation 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 on 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. **FAQ**

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Choline.

19. **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)