ab102531

Trypsin Activity Assay kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Trypsin activity in various samples.

View kit datasheet: www.abcam.com/ab102531
(use www.abcam.cn/ab102531 for China, or www.abcam.co.jp/ab102531 for Japan)

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 12d, Last Updated 26 January 2024

Table of Contents

1. BACKGROUND

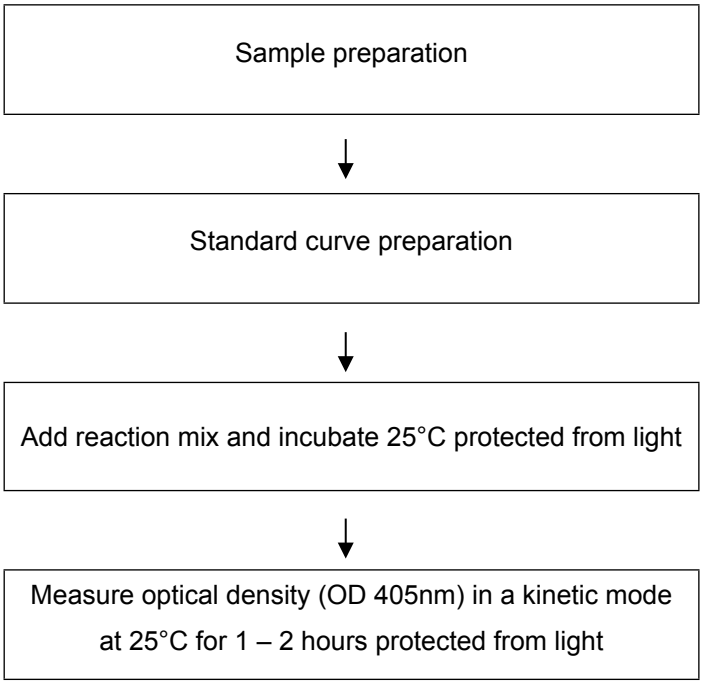
Trypsin Activity Assay Kit (Colorimetric) (ab102531) is an assay where trypsin cleaves a substrate to generate *p*-nitroaniline (*p*-NA) which is detected at OD = 405 nm. Since the color intensity is proportional to *p*-NA content, trypsin activity can be accurately measured.

Trypsin is a serine protease found in the digestive system of many vertebrates, where it hydrolyses proteins. Trypsin is produced in the pancreas as the inactive proenzyme trypsinogen. Active trypsin predominantly cleaves peptide chains at the carboxyl side of the amino acids lysine or arginine, except when either amino acid is followed by proline.

Trypsin is commonly used in numerous biotechnological processes:

- Trypsinization or trypsin proteolysis: digestion of proteins into peptides for mass spectrometry analysis.
- Breakdown casein in break milk.
- Production of hypoallergenic food where proteases break down specific allergenic proteins into nonallergenic peptides (for example, production of hypoallergenic baby food from cow's milk).

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

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/Trypsin Assay Buffer	25 mL	-20°C	4°C
Trypsin Substrate/Trypsin Substrate (in DMSO)	200 µL	-20°C	-20°C
Trypsin Positive Control/Positive Control (2 U, Lyophilized)	1 vial	-20°C	-20°C
p-NA Standard II/*p*-NA Standard (2 mM)	400 µL	-20°C	-20°C
Trypsin Inhibitor/Trypsin Inhibitor (TLCK, 20 mM)	100 µL	-20°C	-20°C
Chymotrypsin Inhibitor II/Chymotrypsin Inhibitor (TPCK,10 mM)	100 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD=405 nm
- 96 well plate: clear plates for colorimetric assay
- Hear block or water bath
- Orbital shaker

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 and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

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

9.1 **Assay Buffer I/Trypsin Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.2 **Trypsin Substrate:**

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 substrate so that you have enough volume to perform the desired number of tests. Store at - 20°C.

9.3 **Trypsin Positive Control/Positive Control (purified Trypsin):**

Reconstitute with 100 µL Assay Buffer I/Trypsin Assay Buffer. Aliquot positive control so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within two months. Keep on ice while in use.

9.4 ***p*-NA Standard II/*p*-NA Standard:**

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 standard so that you have enough to perform the desired number of tests. Store at - 20°C.

9.5 **Trypsin Inhibitor:**

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 inhibitor so that you have

enough volume to perform the desired number of tests. Store at - 20°C.

9.6 **Chymotrypsin Inhibitor II/Chymotrypsin Inhibitor:**

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 inhibitor so that you have enough volume to perform the desires number of tests. Store at - 20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Using 2mM *p*-NA Standard/*p*-NA standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc *p*-NA in well
1	0	150	50	0 nmol/well
2	6	144	50	4 nmol/well
3	12	138	50	8 nmol/well
4	18	132	50	12 nmol/well
5	24	126	50	16 nmol/well
6	30	120	50	20 nmol/well

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

11. SAMPLE PREPARATION

General Sample information:

- This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.
- 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 and spin down briefly.

11.1.3 Resuspend the cell pellet in 4x volumes of Assay Buffer I/Trypsin Assay Buffer, put on ice.

11.1.4 Homogenize using a Dounce homogenizer (10-15 passes) on ice.

11.1.5 Centrifuge 2 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.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg – 100 mg), and cut tissue in small pieces.

11.2.2 Wash tissue in cold PBS.

11.2.3 Add 4x – 6x volumes (500 – 1,000 µL) of Assay Buffer I/Trypsin Assay Buffer and put on ice.

11.2.4 Homogenize using a Dounce homogenizer (10-15 passes) on ice.

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 the supernatant and transfer to a clean tube.

11.3 **Serum and Urine:**

Serum and urine samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

Phenol red and fetal serum present in the culture media will inhibit trypsin activity.

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.
- Positive control = 5 µl Trypsin Positive Control/Positive Control + 45 µL Assay Buffer I/Trypsin Assay Buffer. To ensure consistency, prepare a master mix (for example, mix 15 µL Trypsin Positive Control/Positive Control in 135 µL Assay buffer).
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer I/Trypsin Assay Buffer.
- (Optional) Inhibitor control sample = 2 – 50 µL samples + 1 µL of 50X Trypsin Inhibitor solution (TLCK). Incubate for 5 minutes at room temperature.

12.2 Add 1 µL of Chymotrypsin Inhibitor solution (TPCK) to sample wells and positive control and incubate for 10 minutes at room temperature.

12.3 **Reaction Mix:**

Prepare Reaction Mix for each reaction:

Component	Reaction Mix (µL)
Assay Buffer I/Trypsin Assay Buffer	**48**
Trypsin Substrate	**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)

12.4 Add 50 µL of Reaction Mix into each well of standards, positive controls, samples and inhibitor control sample group (if using).

12.5 Mix and incubate at 25°C protected from light.

12.6 Measure absorbance immediately at OD=405 nm in a kinetic mode, every 2 – 3 minutes, for 1 – 2 hours at 25°C protected from light. Incubate for up to 4 hours if trypsin activity is low.

NOTE: *Sample incubation time can vary depending on Trypsin activity in the samples. We recommend measuring absorbance in kinetic mode and then choosing two time points (T1 and T2) in the linear range to calculate the trypsin activity in the samples.*

For standard curve, do not subtract A_1 from A_2 reading.

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

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of *p*-NA.

13.4 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).

13.5 The color generated by cleavage of substrate is:

$$\Delta A_{405nm} = (A_2 - A_{2C}) - (A_1 - A_{1C})$$

Or if no trypsin inhibitor control was run:

$$\Delta A_{405nm} = (A_2 - A_1)$$

Where:

A1 is the sample reading at time T1.

A1C is the inhibitor control sample at time T1.

A2 is the sample reading at time T2.

A2C is the inhibitor control sample at time T2.

13.6 Substitute A1 and A2 for the p-NA standard curve formula to obtain B1 and B2, respectively. To obtain B nmol of p-NA

generated by Trypsin during the reaction time (ΔT = T2 – T1), subtract B1 from B2 (B=B2-B1).

13.7 Trypsin Activity in the test samples is calculated as:

$$Trypsin\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D = \ nmol/min/ml = mU/ml$$

Where:

B = Amount of p-NA from the Standard Curve (nmol).

ΔT = reaction time (T2 – T1) (min).

V = Amount of pretreated sample volume added to reaction well (in mL).

D = Sample dilution factor.

Unit Definition:

1 Unit = amount of trypsin that cleaves the substrate, yielding 1.0 µmol of *p*-NA per minute at 25°C.

1 *p*-NA Unit (µmol/min) = 0.615 TAME Unit = 35 BAEE Unit.

TAME = p-Toluene-sulfonyl-L-Arginine Methyl Ester

BAEE = Nα-Benzoyl-L-Arginine Methyl Ester

14. TYPICAL DATA

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

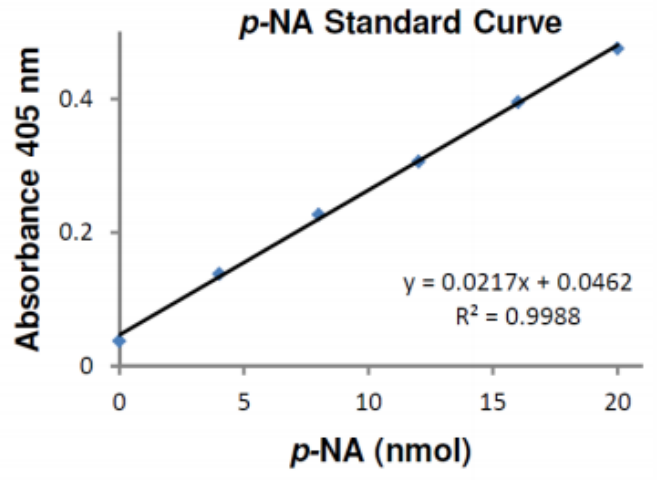


Figure 1: Typical trypsin standard calibration curve generated following assay kit protocol.

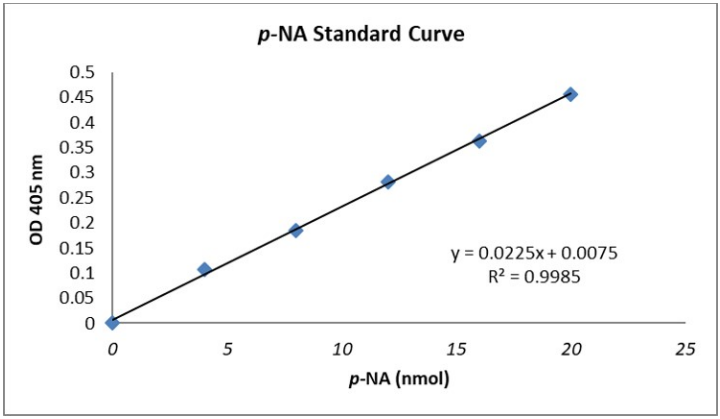


Figure 2: p-NA standard curve measured in duplicates ((+/- SD), background subtracted) after 60 minutes incubation.

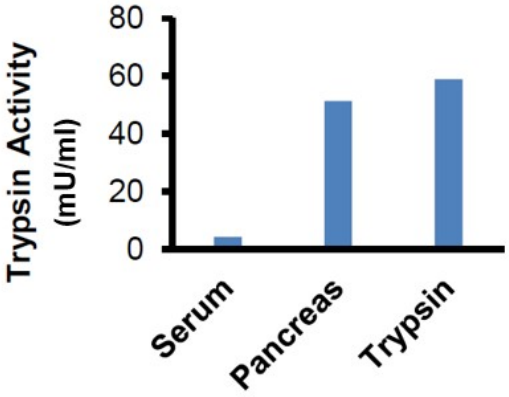


Figure 3: Measurement of Trypsin activity in Human serum (1 µL), Human pancreas lysate (1µL) and commercially available Trypsin (1 µL).

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.*

- Solubilize Trypsin Positive Control/Positive Control, prepare Trypsin Substrate and Trypsin Inhibitor, and Chymotrypsin Inhibitor II/Chymotrypsin Inhibitor, (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples (find optimal dilutions to fit standard curve readings, make up to 50 µL with Assay Buffer).
- Set up plate in duplicate for standard (50 µL), samples (50 µL), positive control (50 µL) and (optional) inhibitor control group (50 µL).
- Prepare Trypsin Reaction Mix (50 µL/well) (Number samples + standards + 1).

Component	Reaction Mix (µL)
Assay Buffer I/Trypsin Assay Buffer	**48**
Trypsin Substrate	**2**

- Add 1 µL of Chymotrypsin (TPCK) solution to samples wells and positive control and incubate for 10 min at room temperature.
- Optional Trypsin Inhibitor control: Add 1 µL of 50X Trypsin Inhibitor to inhibitor control group wells and incubate for 5 min.
- Add 50 µL of Reaction Mix into each well.
- Measure absorbance at OD=405 nm on a microplate reader in a kinetic mode at 25°C for 1 – 2 hours protected from light.

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 described in the 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. FAQs

Should protease inhibitors be added to the buffer?

Trypsin itself is a Serine protease and hence adding protease inhibitors will affect its activity too.

Can a one-time point measurement be done with this assay for the trypsin activity in the sample?

We suggest doing measurements over time intervals because the rate of enzyme activity changes with respect to time. Once the customer has chosen a certain end time point for their experiments, they can do a cumulative one-time point measurement. However, Trypsin activity can be more accurately measured when you do kinetic measurements versus an end-point assay.

Can Trypsin with known activity from another source be used to validate this assay?

If Trypsin is purchased from another vendor and you try to get back the exact activity numbers specified by the vendor using the Abcam assay, it is not going to be possible due to the assay buffer composition and pH differences (between the Abcam Assay buffer and the conditions used by the vendor to define their units of activity).

Can milk be used as a sample in this kit?

Milk can be used with this kit. It is essential to run a pilot experiment using a broad range of dilutions so that the final dilution can be chosen such that the reading is within the linear range of the standard curve. It is essential that samples not be cloudy due to addition of too much milk for accurate OD measurements.

Will phenol red or any other component in media interfere in this assay?

Phenol red might change color at the pH which is optimum for Trypsin activity. Also any serum in the medium inhibits trypsin activity.

Can the activity of the positive control decrease over time?

Trypsin is notoriously well-known for digesting itself in solution leading to decrease in activity over time.

For the inhibitor treatments, should the inhibitor and reaction mix be added and then incubated?

Treat with 1 µL of 50X chymotrypsin inhibitor (TPCK) solution and incubate for 10 minutes at room temperature. Add 1 µL of 50X trypsin inhibitor (TLCK) solution to trypsin inhibitor sample control and incubate for 5 min. These incubations are before adding the reaction mix.

Can a negative control be set up by adding Trypsin inhibitor to the positive control?

Yes, it can be done but we do not suggest inhibiting the purified Trypsin (positive control) to get a negative control. The negative control is based on the inhibition of the trypsin in the sample. A trypsin inhibitor sample group can be setup as a control by adding 1 µl of 50X trypsin inhibitor (TLCK) solution to trypsin inhibitor sample control. Once Trypsin is inhibited, any residual protease activity can be accounted for. Also, there is a Chymotrypsin inhibitor to account for any endogenous chymotrypsin like activity in the sample.

Why do we see the same OD for 25 µL and 50 µL sample?

It is possible that you do not see a dose-dependent relationship if you are adding too much of the enzyme and the readings are very high/saturated. The substrate becomes limiting if there is too much enzyme and can cause such issues. We would suggest diluting in the

assay buffer or simply water and then optimize the sample amount needed in the assay to get values in the linear range of the standard curve.

What is the shelf-life of the positive control if stored unthawed at -20°C or -80°C for 2-3 years?

Trypsin is notoriously well known for its self-destructive protease activity when stored for long periods of time. Once reconstituted, the positive control is to be used within 2 months for best results. Hence we do not expect the positive control enzyme to retain normal activity over 2-3 years even at -20°C or -80°C.

18. INTERFERENCES

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

- Phenol red (present in cell culture medium).
- Fetal or bovine calf serum (as part of cell culture medium).
- RIPA: contains SDS which can destroy/decrease the activity of the enzyme.
- This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.

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)