Version 2a, Last updated 23 August 2023

# ab241009 Chitotriosidase Assay Kit

For the measurement of Chitotriosidase activity in biological 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.

# Table of Contents

# 1. Overview

Chitotriosidase Assay Kit (ab241009) utilizes a fluorogenic substrate that can be hydrolyzed by chitinase and a set of proprietary assay buffers that can distinguish specific CHIT1 activity from other hydrolases including Acidic Mammalian Chitinase. The kit provides a simple, specific, sensitive assay that can detect as low as 0.2 mU/mL of CHIT1 in a variety of biological samples.


Prepare samples, sample controls, inhibition samples, inhibition control samples, positive control and standards as described.

⇩

Initiate reaction by addition of diluted Chitinase Substrate/CHIT1 substrate (50 µl) to samples, inhibition samples and positive control. Add Assay Buffer LI/CHIT1 Assay Buffer (50 µl ) to sample controls and sample inhibition controls.

⇩

Incubate plate at 37 °C for 20 -30 minutes.

⇩

Read in kinetic mode (standards should be read in end-point mode) at Ex/Em = 320/445 nm).

## 2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

| Item | Quantity | Storage temperature |
|---|---|---|
| Assay Buffer LI/CHIT1 Assay Buffer | 25 mL | -20°C |
| CHIT1 Inhibition Buffer | 18 mL | -20°C |
| Chitinase Substrate/CHIT1 Substrate (in DMSO) | 25 µL | -20°C |
| Chitotriosidase | 1 vial | -20°C |
| 4-Methylumbelliferone Standard/4-Methylumbelliferone Standard (5 mM) | 35 µL | -20°C |

## 3. 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 fluorescence at Ex/Em = 320/445 nm
- 96 well white opaque plate
- Dounce homogenizer (if using tissue)

## 4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

## 5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 5.1 Assay Buffer LI/CHIT1 Assay Buffer

Ready to use as supplied. Store at -20°C or 4°C. Equilibrate to 37°C before adding to assay wells.

### 5.2 CHIT1 Inhibition Buffer

Ready to use as supplied. Store at -20°C or 4°C.

### 5.3 Chitinase Substrate/CHIT1 Substrate (in DMSO)

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

### 5.4 4-Methylumbelliferone Standard/4-Methylumbelliferone Standard (5mM)

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

### 5.5 Chitotriosidase

Reconstitute Chitotriosidase in 55 µL Assay Buffer LI/CHIT1 Assay Buffer and mix thoroughly. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.

## 6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 100 µM solution of 4-Methylumbelliferone (4-MU) by adding 5 µL of 5 mM 4-MU to 245 µL Assay Buffer LI/CHIT1 Assay Buffer.
2. Further dilute the 100 µM Standard Solution by adding 20 µL of 100 µM to 180 µL Assay Buffer LI/CHIT1 Assay Buffer to generate a 10 µM 4-MU Standard.
3. Add 0, 10, 20, 30 and 40 µL of 10 µM 4-MU standard into a series of wells to generate 0, 100, 200, 300 and 400 pmol of 4-MU/well respectively. Adjust the volume to 100 µL/well with Assay Buffer LI/CHIT1 Assay Buffer.

| Standard # | 10 µM 4-MU Standard (µL) | Assay Buffer LI/CHIT1 Assay Buffer (µL) | Final volume standard in well (µL) | 4-MU Standard (pmol/well) |
|---|---|---|---|---|
| 1 | 0 | 100 | 100 | 0 |
| 2 | 10 | 90 | 100 | 100 |
| 3 | 20 | 80 | 100 | 200 |
| 4 | 30 | 70 | 100 | 300 |
| 5 | 40 | 60 | 100 | 400 |

# 7. 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 for the most reproducible assay.

### 7.1 Cells or tissues:

1. Divide cell pellet or tissue samples into 2 tubes (~1 X $10^6$ cells each or 5-20 mg tissue each). Homogenize each tube containing cells/tissue with 100 µL of ice cold Assay Buffer LI/CHIT1 Assay Buffer and 100 µL of ice cold CHIT1 Inhibition Buffer, respectively.

**Δ Note:** We recommend both buffers should contain protease inhibitor cocktail.

2. Keep samples on ice for 10 min. Centrifuge samples at 12,000 x *g* at 4 °C for 5 minutes and collect the supernatants, separately.

**Δ Note:** Do not pool these samples, keep them in different, pre-labeled tubes.

3. Record final volume and protein concentration of each homogenate.
4. Add 5-20 µL of each sample into 2 wells: the Assay Buffer LI/CHIT1 Assay Buffer homogenates should have 2 wells: Sample [S], Sample Control [SC]; the CHIT1 Inhibition Buffer homogenates should have 2 wells: Inhibition Sample [IS], Inhibition Sample Control [ISC]. Use the protein concentration of each in the calculations below

### 7.2 Biological fluids:

1. Divide Biological Fluids into 2 tubes. Adjust to pH 2.0 in one tube using a 5-fold dilution of Biological Fluids in CHIT1 Inhibition Buffer (i.e. dilute 5 µL of sample with 20 µL of CHIT1 Inhibition Buffer). Record the added volume (ΔV).
2. Add the same volume (ΔV) of Assay Buffer LI/CHIT1 Assay Buffer to the second test tube. Both samples should be diluted in the same fashion.
3. Add 1-20 µL of samples prepared in Assay Buffer LI/CHIT1 Assay Buffer into 2 parallel wells: Sample [S], Sample Control [SC]; add

the same volume of samples prepared in CHIT1 Inhibition Buffer into 2 parallel wells: Inhibition Sample [IS], Inhibition Sample Control [ISC]. Use the dilution factor in the calculations below.

### 7.3 Chitotriosidase positive control:

1. Add 2-4 µl of reconstituted Chitotriosidase into desired well(s).

# 8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

**Δ Note:** For unknown samples, we recommend doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range. Do not use more than 20 µL of sample in each well.

## 8.1 Chitinase Substrate/CHIT1 substrate solution:

1. Prepare a 625-fold dilution of Chitinase Substrate/CHIT1 Substrate Stock Solution (i.e. Dilute 1 µL of Chitinase Substrate/CHIT1 Substrate with 624 µL of Assay Buffer LI/CHIT1 Assay Buffer), vortex briefly.
2. Add 50 µL of Diluted Chitinase Substrate/CHIT1 Substrate Solution to each well containing test Sample [S], Inhibition Sample [IS] and CHIT1 positive control; Add 50 µL of Assay Buffer LI/CHIT1 Assay Buffer to wells assigned as Sample Control [SC] and Inhibition Sample Control [ISC].

**Δ Note:** Equilibrate Assay Buffer LI/CHIT1 Assay Buffer to 37 °C before adding to the assay wells.

**Δ Note:** Equilibratesubstrate solutions to 37 °C before adding to the assay wells.

## 8.2 Measurement:

1. Measure fluorescence (Ex/Em 320/445nm) of samples and standards in kinetic mode at 37 °C for 20-30 minutes and endpoint settings, respectively.

**Δ Note**: : Incubation time depends on the CHIT1 activity in samples. Longer incubation time may be required for samples having low CHIT1 activity.

## 9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Substrate 0 Standard reading from all standard readings. Plot the 4-MU Standard Curve.
3. For each reaction well, choose two time points ($t_1$ and $t_2$) in the linear range of the plot, obtain the corresponding fluorescence values ($RFU_1$ and $RFU_2$), apply sample $\Delta$RFU to the 4-MU Standard Curve to obtain the corresponding pmol of product formed (B, in pmol).

$$\text{Activity in sample well (A)} = \frac{B}{(V * \Delta t)} * D$$

Where:

**A** = Chitotriosidase activity (µU/mL).

**B** = 4-MU from standard curve (pmol).

**$\Delta$t** = reaction time (minutes).

**D** = sample dilution factor.

4. Subtract the activity value of the background control from test samples (such as: $\Delta SC = A_{[S]} - A_{[SC]}$ ; $\Delta ISC: A_{[IS]} - A_{[ISC]}$) to determine the background-corrected change in enzymatic activity for each sample or sample with inhibition. Calculate CHIT1 activity by subtracting the background-corrected sample with inhibition from background-corrected each sample.

**Sample CHIT1** = [$\Delta SC - \Delta ISC$] (pmol/minute/mL) = µU/mL

= [($A_{[S]} - A_{[SC]} - (A_{[IS]} - A_{[ISC]})$] (pmol/minute/mL) = µU/mL

**Unit definition:** One unit of CHIT1 activity is the amount of enzyme that generate 1.0 µmol of 4-MU per min., at pH 4.2 at 37 °C. CHIT1 specific activity can be expressed as U/mg of protein or 1 nmol/h.ml (16.7 µU/ml).

## 10. Typical Data

Data provided **for demonstration purposes only**.

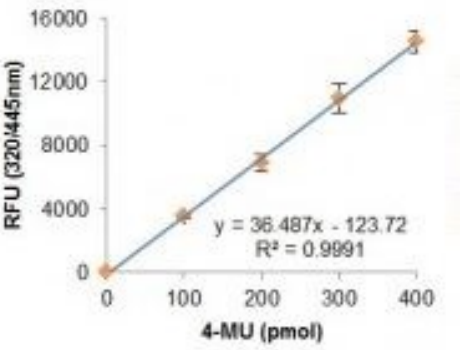


**Figure 1**. 4-Methylumbelliferon Standard Curve.

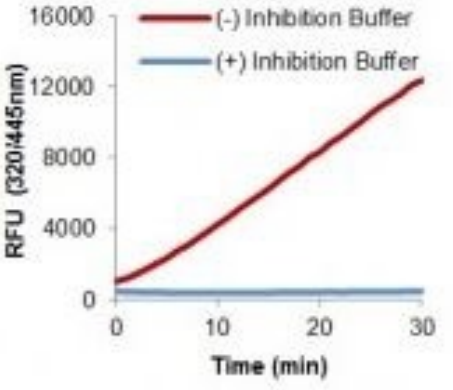


**Figure 2**. Measurement of purified Human Chitiotriosidase activity with or without Inhibition Buffer.

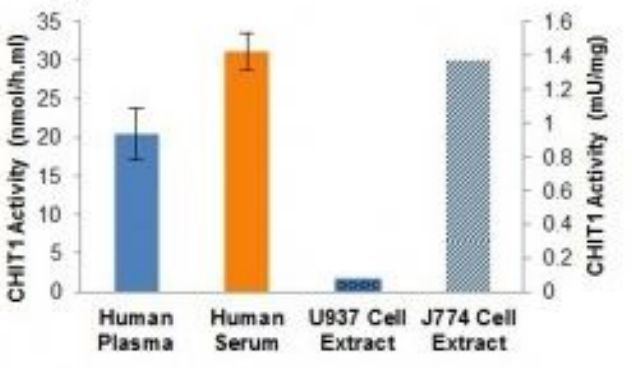


**Figure 3.** Measurement of CHIT1 activity in human plasma (4 µL), human serum (4 µL, U937 cell extract (10 µg) and J774 cell extract (10 µg).

# 11. 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)