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Chitotriosidase Activity Assay Kit (Fluorometric)

06/16

(Catalog # K512-100; 100 assays; Store at -20°C)

I. Introduction:

Chitotriosidase (CHIT1 or chitinase-1), is a mammalian enzyme belonging to glycosyl hydrolase-18 family (EC 3.2.1.14), which can hydrolyze the β-(1, 4)-linkage between the adjacent *N*-acetyl glucosamine residues of chitin. CHIT1 is synthesized exclusively by activated macrophages both in normal and inflammatory conditions and, as a host defense factor, CHIT1 plays a pivotal role in infectious disease such as malaria and fungi infectious. Increased CHIT1 activity has also been found in patients suffering diseases such as bronchial asthma and atherosclerosis. CHIT1 has been used as a valuable diagnostic biomarker for monitoring the therapeutic efficacy of treatments for Gaucher's disease or β-glucocerebrosidase deficiency. BioVision's Chitotriosidase Activity Assay, 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.

CHIT1 substrate $\xrightarrow{\text{Chitotriosidase}}$ Cleaved substrate + Fluorescent product (Ex/Em=320/445nm)

II. Applications:

- Cell culture: e.g. J774, U937
- Biological fluids: e.g. Serum, Plasma
- Tissue homogenates: Lung, spleen, liver, etc.
- Purified enzyme preparations

III. Kit Contents:

Components	K512-100	Cap Code	Part Number
CHIT1 Assay Buffer	25 ml	WM	K512-100-1
CHIT1 Inhibition Buffer	18 ml	NM	K512-100-2
CHIT1 Substrate (in DMSO)	25 µl	Red	K512-100-3
Chitotriosidase (lyophilized)	1 vial	Green	K512-100-4
4-Methylumbelliferone Standard (5 mM)	35 µl	Yellow	K512-100-5

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate
- Multi-well spectrophotometer (fluorescence plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **CHIT1 Assay Buffer and CHIT1 Inhibition Buffer:** Store at either 4ºC or -20ºC.
- **CHIT1 Substrate and 4-Methylumbelliferon Standard:** Store at -20ºC. Bring to room temperature before use.
- **Chitotriosidase:** Reconstitute Chitotriosidase in 55 µl ***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.

VI. Chitotriosidase Assay Protocol:

1. Sample Preparation:

a) For Cells or tissues: 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 **CHIT1 Assay Buffer** and 100 µl of ice cold **CHIT1 Inhibition Buffer** respectively. We recommend both buffers should contain protease inhibitor cocktail (Cat. # K272 or equivalent). Keep samples on ice for 10 min. Centrifuge samples at 12,000 x g at 4 ºC for 5 min. and collect the supernatant, separately; *Do not pool these samples, keep them in different, pre-labeled tubes*. Record final volume and protein concentration of each homogenate. Add 5-20 µl of each sample into 2 wells: the **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 (Step 5).

b) For Biological Fluids: 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). Add same volume (ΔV) of **CHIT1 Assay Buffer** to the second test tube. *Both samples should be diluted in the same fashion*. Add 1-20 µl of samples prepared in **CHIT1 Assay Buffer** into 2 parallel wells: Sample [S], Sample Control [SC]; add same volume of samples prepared in **CHIT1 Assay Buffer** into 2 parallel wells: Inhibition Sample [IS], Inhibition Sample Control [ISC]. Use the dilution factor in the calculations below (Step 5).

c) For positive control, add 2-4 µl of Chitotriosidase into desired well(s).

Adjust the volume of Positive Control, Sample and Sample Control, Inhibition Sample and Inhibition Sample Control wells to 50 µl/well with **CHIT1 Assay Buffer.**

Notes:

a. 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**.

b. Equilibrate the CHIT1 Assay Buffer to 37 ºC before adding to the wells.

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone (4-MU) by adding 5 µl of 5 mM 4-MU to 245 µl CHIT1 Assay Buffer. Further dilute the 100 µM Standard Solution by adding 20 µl of 100 µM to 180 µl CHIT1 Assay Buffer to generate a 10 µM 4-MU Standard. Add 0, 10, 20, 30, 40 µl of 10 µM 4-MU standard into a series of wells to generate 0, 100, 200, 300, 400 pmol of 4-MU/well respectively. Adjust the volume to 100 µl/well with CHIT1 Assay Buffer.
3. **CHIT1 Substrate Solution Preparation:** Prepare a 625-fold dilution of CHIT1 Substrate Stock Solution (i.e. Dilute 1 µl of CHIT1 Substrate with 624 µl of CHIT1 Assay Buffer), vortex briefly. Add 50 µl of Diluted CHIT1 Substrate Solution to each well containing test Sample [S], Inhibition Sample [IS] and CHIT1 positive control; Add 50 µl of CHIT1 Assay Buffer to wells assigned as Sample Control [SC] and Inhibition Sample Control [ISC].

   Note: Equilibrate the Substrate Solutions to 37 ºC before adding to the wells
4. **Measurement:** Measure fluorescence (Ex/Em 320/445nm) of samples and standards in kinetic mode at 37 ºC for 20-30 min. and end-point settings respectively.

   Note: Incubation time depends on the CHIT1 activity in samples. Longer incubation time may be required for samples having low CHIT1 activity.
5. **Calculation:** Substrate 0 Standard reading from all standard readings. Plot the 4-MU Standard Curve. 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 ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (B, in pmol). Calculate the enzymatic activity in each well (A) as:

Activity in each sample well (A) = B/ (Δt * V) * D = pmol/min/ml = µU/ml

Where: **B** = 4-MU from Standard Curve (pmol)
Δt = Reaction time (min.)
V = Sample volume added into the reaction well (ml)
D = Dilution factor

Subtract the activity value of the background control from test samples (such as: **ΔSC:** $A_{[S]}$ - $A_{[SC]}$; **ΔISC:** $A_{[IS]}$ - $A_{[ISC]}$) 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= [ΔSC- ΔISC] (pmol/min/ml = µU/ml)

= [($A_{[S]}$ - $A_{[SC]}$) - ($A_{[IS]}$ - $A_{[ISC]}$)] (pmol/min/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 ºC. CHIT1 specific activity can be expressed as U/mg of protein or 1 nmol/h.ml (16.7 µU/ml).

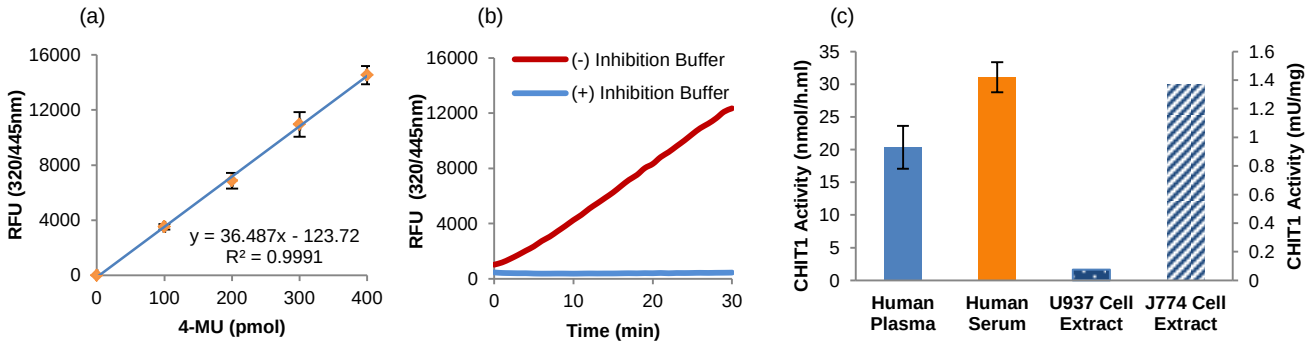

Figure: (a) 4-Methylumbelliferon Standard Curve. (b) Measurement of purified Human Chitiotriosidase activity with or without Inhibition Buffer. (c) Measurement of CHIT1 activity in Human Plasma (4 µl), Human Serum (4 µl), U937 Cell Extract (10 µg) and J774 Cell Extract (10 µg). All assays were performed following kit protocol.

VII. RELATED PRODUCTS:

Lysozyme Activity Assay Kit (K236)
Acidic Mammalian Chitinase Activity Kit (K513)
Lysozyme Inhibitor Screening Kit (K237)
Acidic Mammalian Chitinase Inhibitor Screening Kit (693)

FOR RESEARCH USE ONLY! Not to be used on humans.