Technical Bulletin

Chitinase Assay Kit

Catalogue number CS0980

Product Description

Chitinase catalyzes the hydrolytic cleavage of the β(1→4)-glycoside bonds present in biopolymers of N-acetylglucosamine, primarily in chitin. Chitinases are widely distributed in living organisms and are found in fungi, bacteria, parasites, plants, and animals.[1] They are classified in families based on amino acid sequence similarities.[2]

The chitinolytic enzymes are also categorized based on their enzymatic action on chitin substrates. Endochitinases are defined as the enzymes catalyzing the random cleavage at internal points in the chitin chain. Exochitinases catalyze the progressive release of acetylchitobiose or N-acetylglucosamine from the non-reducing end of chitin, and thus, are referred to as chitobiosidase and β-N-acetylglucosaminidase, respectively.[3,4]

Chitinases perform different functions in different organisms. In bacteria they are mainly involved in nutritional processes, while in yeast and various fungi, these enzymes participate in morphogenesis. In animals and plants, chitinases primarily play a role in the defense of the organism against pathogen attack.

Human chitotriosidase (Chit), a chitionolytic enzyme, is a member of the chitinase family. In human plasma, Chit activity has been proposed as a biological marker of macrophage activity in several lysosomal diseases, and was found at higher levels in patients with *Plasmodium falciparum* malaria infection. This suggests that Chit induction may reflect an immunological response.[5]

Another member of the chitinase family is the acid mammalian chitinase (AMCase), thought to play a role in inflammatory disorders. Elevated levels of ACMase were observed in lung tissue of asthmatic patients, suggesting a role for this enzyme in asthma pathophysiology.[6]

The kit assay is based on the enzymatic hydrolysis of chitinase substrates. This hydrolysis releases *p*-nitrophenol (4-nitrophenol), which upon ionization in basic pH, can be measured colorimetrically at 405 nm.[7]

The Chitinase Assay Kit provides all the reagents required for efficient detection of chitinase activity in fungal and bacterial growth media, macrophage lysates, and purified enzyme preparations. In addition, the kit provides three different substrates for the detection of the various types of the chitinolytic activity:[3]

4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside – a substrate suitable for exochitinase activity detection (chitobiosidase activity)

4-Nitrophenyl N-acetyl-β-D-glucosaminide – a substrate suitable for exochitinase activity detection (β-N-acetylglucosaminidase activity)

4-Nitrophenyl β-D-N,N′,N′′-triacetylchitotriose – a substrate suitable for endochitinase activity detection

The kit was tested on *Trichoderma viride*, *Streptomyces griseus*, and human macrophages.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalogue Number A4855
- 4-Nitrophenyl N-acetyl-β-D-glucosaminide 10 mg
  Catalogue Number AKB25
- 4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside 5 mg
  Catalogue Number N6133
- 4-Nitrophenyl β-D-N,N′,N′′-triacetylchitotriose 1 mg
  Catalogue Number N8638
- Chitinase from *Trichoderma viride* 1 mg
  Catalogue Number C6242
- *p*-Nitrophenol Solution, 10 mM 1 mL
  Catalogue Number N7660
- Sodium Carbonate 1 g
  Catalogue Number S2127

The kit is sufficient for the number of multiwell plate reactions indicated for each substrate:

AKB25 at a substrate concentration of 1 mg/ml is sufficient for 100 reactions.

N6133 at a substrate concentration of 1 mg/ml is sufficient for 50 reactions.

N8638 at a substrate concentration of 1 mg/ml is sufficient for 10 reactions.

Note: For other substrate concentrations see Substrate Solution(s) Notes under Preparation Instructions.

Equipment Required but Not Provided

- Spectrophotometer plate reader (405 nm)
- 96 well plates (flat bottom, Catalogue Number P7366)
- 37 °C water bath
- Dulbecco's Phosphate Buffered Saline (PBS, Catalogue Number D8537)
- For macrophages lysis: CelLytic™ M Cell Lysis Reagent (Catalogue Number C2978)

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C is recommended. Upon arrival it is recommended to store the NP-GlcNAc (Catalogue Number AKB25) and the Chitinase from *Trichoderma viride* (Catalogue Number C6242) at -20 °C and the Sodium Carbonate (Catalogue Number S2127) at room temperature.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Stop Solution (sodium carbonate solution) – Add 24 ml of ultrapure water to the contents of the sodium carbonate bottle (Catalogue Number S2127) and mix well with a magnetic stirrer until completely dissolved. Store the Stop Solution at room temperature.

Substrate Solution(s) (1 mg/ml) – Dissolve 1 mg of the appropriate substrate in 1 ml of Assay Buffer (Catalogue Number A4855):

4-Nitrophenyl N-acetyl-β-D-glucosaminide,
Catalogue Number AKB25, or

4-Nitrophenyl β-D-N,N′,N′′-triacetylchitotriose,
Catalogue Number N8638 or

4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside,
Catalogue Number N6133

1 ml of Substrate Solution is sufficient for ~10 reactions. Mix the Substrate Solution(s) with rocking/shaking on a horizontal shaker at room temperature.

Notes: A 1 mg/ml substrate solution is recommended. If required, the 4-Nitrophenyl β-D-N,N′,N′′-triacetyl-chitotriose and the 4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside Substrate Solutions can be used at lower concentrations in the reaction (0.5 or 0.2 mg/ml, respectively).

The substrates do not dissolve easily in the buffer. It may take ~1 hour of shaking to completely dissolve the substrates. Use of a larger container (15 ml) may aid dissolution. The Substrate Solution(s) should be stored on ice during the experiment. For long term storage, up to one month, store at –20 °C.

Chitinase Control Enzyme – Add 5 ml of PBS to the contents of the chitinase bottle (Catalogue Number C6242) to give a final chitinase concentration of 0.2 mg/ml. Vortex until dissolved. The chitinase dissolves immediately to give a slightly hazy solution. For long term storage, store in working aliquots at –20 °C (stable for at least 3 months at –20 °C). Just before use, dilute the chitinase 20-fold with PBS and keep on ice.

Standard Solution – Before performing the assay in 96 well plates, dilute 5 µl of the 10 mM *p*-Nitrophenol Solution (Catalogue Number N7660) with 995 µl of Stop Solution. Vortex briefly and store on ice.

Sample Preparation – *Trichoderma viride* and *Streptomyces griseus* growth medium can be sampled directly from the growing culture (since the chitinase is secreted into the growth medium) and can be used in the assay after a brief centrifugation to remove particles from the medium. Human macrophage proteins can be extracted with CelLytic M Cell Lysis Reagent (Catalogue Number C2978).

Procedure

The chitinase hydrolysis is performed in an acidic environment (pH ~4.8) at 37 °C.[6] The enzymatic hydrolysis liberates *p*-nitrophenol.[8] Addition of the basic Stop Solution causes ionization of the *p*-nitrophenol to form the yellow *p*-nitrophenylate ion. The absorbance of the *p*-nitrophenylate ion is measured at 405 nm.[3]

In order to quantitate the total chitinolytic activity, separate reactions should be run with the three substrates supplied in the kit. Profiling of the chitinolytic enzymes can be determined after separation of the chitinolytic enzymes by SDS-PAGE, using an agarose overlay containing fluorescent substrates.[3,9] Note that in crude preparations there

may be additive/synergist activity of different chitinases.

It is recommended to perform the assays in duplicates. For each substrate, perform a separate activity assay according to the following instructions.

Assay Reaction

1. Equilibrate the Substrate Solution(s) and the Standard Solution to 37 °C by incubating for several minutes in a 37 °C water bath.

2. Set the plate reader at 405 nm.

3. Add the reaction components to the 96 well plate according to Table 1 and mix using a horizontal shaker or by pipetting. The substrate should be added first, and the enzyme should be added last.

Table 1.
Reaction Scheme for 96 Well Plate Assays

	Substrate Solution	Sample	Standard Solution
Blank*	100 µl	–	–
Standard**	–	–	300 µl
Positive control***	90–99 µl	1–10 µl of Chitinase Control Enzyme	–
Test	90–99 µl	1–10 µl of sample	–

* A blank reaction (Substrate Solution without enzyme) should be run, since a portion of the substrate may hydrolyze spontaneously during the incubation time.

** A standard should be run when activity calculations are required.

*** The volume of the enzyme can range between 1–10 µl, depending on the reaction duration (i.e., for a shorter time a higher volume of the enzyme is required). The positive control enzyme is the 20-fold diluted enzyme. If required, the concentrated, non-diluted enzyme may be used.

4. Incubate the plate for 30 minutes at 37 °C. If required, the incubation time for highly active samples can be reduced to as low as 5 minutes.

5. Stop the reactions by adding 200 µl of Stop Solution to each well, except for the wells containing the Standard Solution. After the addition of the Stop Solution the reaction mixture will develop a yellow tint.

6. Measure the absorption at 405 nm no later than 30 minutes after ending the reaction.

Results

Calculations

Unit definition: One unit will release 1.0 µmole of *p*-nitrophenol from the appropriate substrate per minute at pH 4.8 at 37 °C.

$$\text{Units/ml} = \frac{(A_{405}\text{sample} - A_{405}\text{blank}) \times 0.05 \times 0.3 \times DF}{A_{405}\text{standard} \times \text{time} \times V_{enz}}$$

A_{405}sample – absorbance of the sample at 405 nm

A_{405}blank – absorbance of the blank at 405 nm

0.05 – µmole/ml of *p*-nitrophenol in the Standard Solution

0.3 – final volume of the 96 well plate reaction after addition of the Stop Solution (ml)

DF - Dilution Factor - fold dilution of the original chitinase enzyme or biological solution to prepare sample for the test

A_{405}standard – absorbance of the Standard Solution at 405 nm

time – minutes

V_{enz} – volume of the sample (ml)

References

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