ELISA PRODUCT INFORMATION & MANUAL

Chymotrypsin Activity Assay Kit (Fluorometric)

NBP2-59744

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

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1. Overview

Chymotrypsin Activity Assay Kit (Fluorometric) (NBP2-59744) uses a synthetic fluorogenic substrate, enabling kinetic measurement of chymotrypsin activity in cell and tissue lysates. A chymotrypsin activator cleaves chymotrypsinogen to form active chymotrypsin, which then hydrolyzes the non-fluorescent substrate to release a stable fluorophore. The kit includes a selective chymotrypsin inhibitor that can be used to measure specific chymotrypsin activity in samples containing non-specific proteases and endopeptidases that may also metabolize the substrate. The assay can detect as low as 0.01 mU of Chymotrypsin.

Homogenize sample with ice-cold Chymotrypsin Assay Buffer and keep on ice for 10 minutes.

Prepare sample wells and Chymotrypsin Positive Control well and add Chymotrypsin Assay Buffer for background control well.

Generate a Standard Curve using the Coumarin Standards Stock solution.

Prepare reaction mixes for test sample and sample background control wells. Add sample background control mix to each of the sample background control wells and add reaction mix to wells containing samples, sample + inhibitor, positive control and reagent background control.

Record the fluorescence Ex/Em= 380/460 nm in kinetic mode for 30-60 minutes at 25 °C.

2. Materials Supplied and Storage

Store kit at -20°C protected from light 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.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Chymotrypsin Assay Buffer	25 mL	-20°C	-20°C
Chymotrypsin Substrate	200 µL	-20°C	-20°C
Chymotrypsin Activator	1 vial	-20°C	-80°C
Chymotrypsin Inhibitor	80 µL	-20°C	-20°C
Coumarin Standard (1 mM)	100 µL	-20°C	-20°C
Chymotrypsin Positive Control	1 vial	-20°C	-80°C

3. Materials Required, Not Supplied

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

- 96-well clear or white plate with flat bottom.
- Multi-well spectrophotometer.
- Anhydrous DMSO.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

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 Chymotrypsin Assay Buffer

1. Warm to room temperature before use.

5.2 Chymotrypsin Substrate

1. Thaw completely before use.
2. Mix well.

5.3 Chymotrypsin Activator

1. Reconstitute with 220 µL Chymotrypsin Assay Buffer immediately before use.
2. Aliquot remainder and store at -80 °C.
3. Once reconstituted, use within 2 months.

5.4 Chymotrypsin Inhibitor

1. Thaw completely before use.
2. Mix well.

5.5 Coumarin Standard

1. Thaw completely before use.
2. Mix well.

5.6 Chymotrypsin Positive Control

1. Reconstitute with 22 µL Chymotrypsin Assay Buffer immediately before use.
2. Aliquot remainder and store at -80 °C.
3. Once reconstituted, use within 2 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. Add 0, 2, 4, 6, 8 and 10 µL from the provided 1 Mm Coumarin Standard stock solution into a series of wells in a clear 96-well plate.
2. Bring the total volume up to 100 µL per well with Chymotrypsin Assay Buffer to generate 0, 2, 4, 6, 8 and 10 nmol/well of Coumarin Standard.
3. Mix by pipetting, making sure that no bubbles are introduced in the wells.

Δ Note: If sample activity is low (outside standard curve RFU values), another standard curve ranging from 0.1 to 1 nmol/well may be generated. For this, dilute the provided Coumarin Standard 1:10 in DMSO to obtain a 100 µM Coumarin Standard solution. Add 0, 2, 4, 6, 8 and 10 µL of the 100 µM solution into a series of wells in a 96 well plate and bring the total volume up to 100 µL with Chymotrypsin Assay Buffer to generate 0, 0.2, 0.4, 0.8 and 1 nmol/well of Coumarin Standard.

7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

1. Homogenize cells (1 x 10^6) or tissue (20 mg) with 100 µL ice-cold Chymotrypsin Assay Buffer and keep on ice for 10 minutes.
2. Centrifuge at 10,000 x *g* for 10 minutes at 4°C and transfer the supernatant to a fresh tube.
3. Determine protein concentration.
   Δ Note: Protein concentration should range between 5-20 mg/mL. Concentrated samples may be diluted with Chymotrypsin Assay Buffer. Aliquot and store lysates at -80°C unless being used immediately.
4. Use 5-20 µL sample per well using a clear 96-well plate.
5. Prepare two identical wells for each sample labeled "Sample Background Control" and "Sample".
6. An additional well called "Sample + Inhibitor" may be prepared for samples in which nonspecific chymotrypsin-like protease activity is likely to be present. For this, add 2 µL of chymotrypsin inhibitor in addition to sample.
7. Adjust volume in each well to 50 µL with Chymotrypsin Assay Buffer.
   Δ Note: For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

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.

8.1 Positive Control

1. Add 1-4 µL of Chymotrypsin Positive Control into desired well(s) and adjust the final volume to 50 µL with Chymotrypsin Assay Buffer.

8.2 Reagent background control

1. Add 50 µL of Chymotrypsin Assay Buffer to a well.

8.3 Reaction Mix

1. Mix enough reagents for the number of assay to be performed (50 µL/well).
2. Add 50 µL of the Sample Background Control Mix to each of the "Sample Background Control" well and 50 µL of the Reaction Mix to wells containing samples, sample + inhibitor, positive control and reagent background control for a final volume of 100 µL per well.
   Δ Note: Turbidity upon addition of Chymotrypsin Substrate to Chymotrypsin Assay buffer is normal and will disappear following vortexing.

Component	Reaction Mix (µL)	Sample Background Control Mix (µL)
Chymotrypsin Assay Buffer	46 µL	48 µL
Chymotrypsin Substrate	2 µL	-
Chymotrypsin Activator	2 µL	2 µL

8.4 Measurement

1. Immediately start recording the fluorescence (Ex/Em = 380/460 nm) in kinetic mode (i.e. at 30 second intervals) for 30-60 minutes at 25°C.

   Δ Note: Ideal measurement time depends on the chymotrypsin activity in samples. We recommend running the assay in kinetic mode to ensure that the linear reaction phase is recorded. The Coumarin Standard curve can be read in endpoint mode.

9. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (the 0 pmol/well reading) from all other Coumarin standards. This is the corrected absorbance.
3. Plot the standard curve.
4. For sample reaction wells (including paired inhibitor control wells), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curve, obtain the corresponding fluorescence values at those points (RFU_1 and RFU_2) and determine the change in fluorescence over the time interval: $\Delta F = RFU_2 - RFU_1$.
5. Subtract the reagent background control ΔF value from the respective sample ΔF values.
   Δ Note: If sample background control ΔF values are higher than reagents background control, subtract the sample background control from the corresponding sample (rather than subtracting the reagent background control).
6. Chymotrypsin specific activity is obtained by applying the background-corrected ΔF values to the Coumarin Standard curve to get B pmol of substrate metabolized during the reaction time.

$$\text{Chymotrypsin Specific Activity} = \frac{\Delta B}{(\Delta t * p)} = \ = \text{pmol/min/mg} = \mu\text{U/mg}$$

Where:

ΔB = change in Coumarin concentration during reaction (in pmol)

$\Delta t = t_2 - t_1$ (in min)

p = sample protein content added to well (in mg)

If Chymotrypsin inhibitor is being used, calculate chymotrypsin activity as follows:

$$Chymotrypsin\ Activity = Total\ activity\ in\ sample - Activity\ with\ Chymotrypsin\ Inhibitor$$

Unit Definition: One unit of Chymotrypsin is the amount of enzyme that generates 1.0 µmol of coumarin per minute at pH 8 at 25°C

10. Typical Data

Data provided **for demonstration purposes only**.

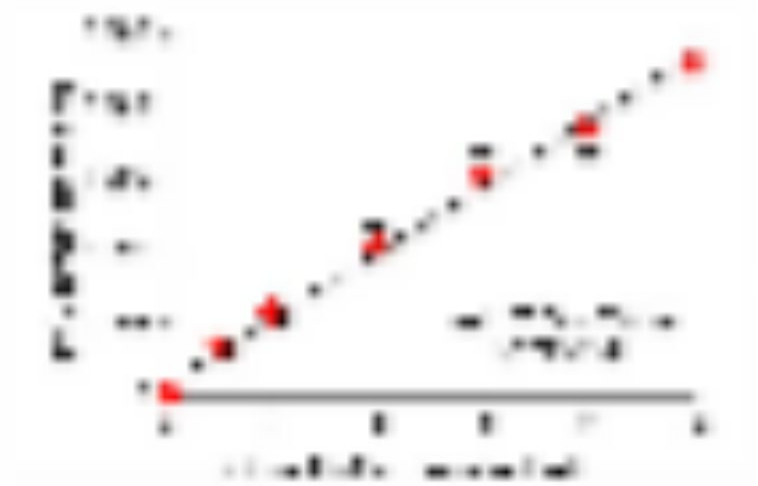

Figure 1. Coumarin standard curves showing a range of 0-10 nmol/well.

Figure 2. Reaction kinetics for positive control and rat pancreatic lysate (9 µg).

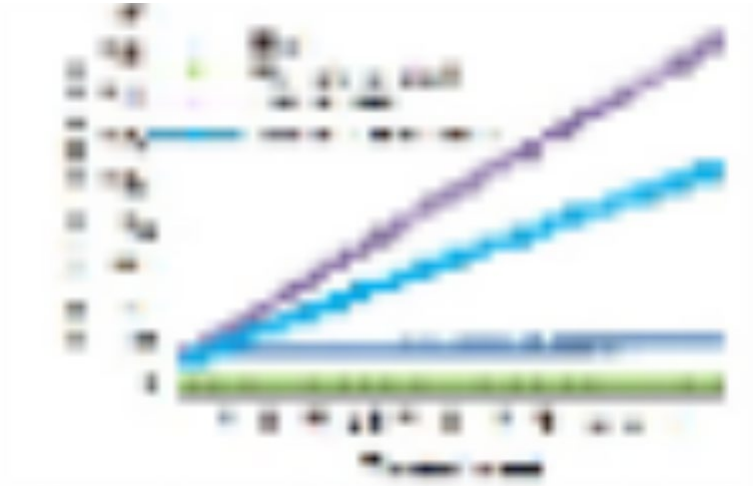

Figure 3. Reaction kinetics for rat spleen lysate (160 µg).

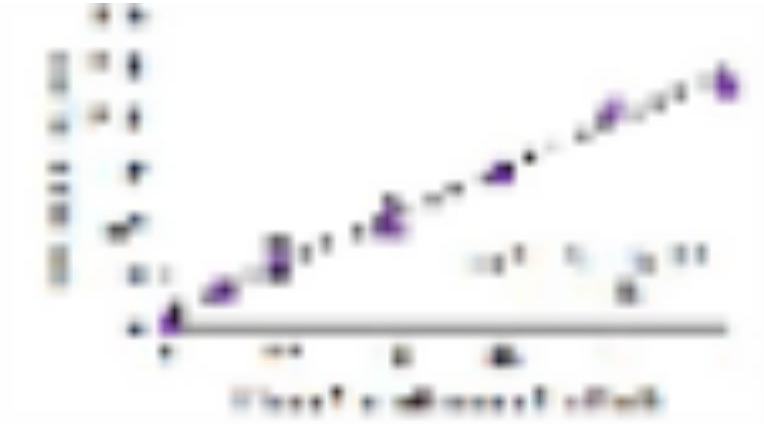

Figure 3. Coumarin standard curves showing a range od 0-1 nmol/well.

Figure 4. Chymotrypsin activity with and without inhibitor in rat pancreas and rat spleen. The presence of non-specific chymotrypsin-like proteases in spleen leads to some activity in presence of the selective chymotrypsin inhibitor.

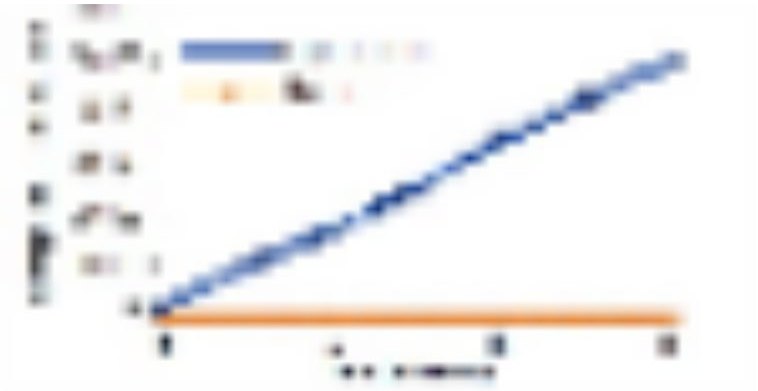

Figure 5. Reaction kinetics using substrate in the presence of Chymotrypsin or Trypsin. The substrate is cleaved by chymotrypsin but not trypsin, making the assay free from trypsin interference.

11. Notes