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Product Information

SensiZyme Cathepsin K Activity Assay Kit

Catalog Number **CS1150**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cathepsin K is a cysteine protease, highly expressed in osteoclasts. It has the ability to catabolize proteins like osteonectin, elastin, collagen, and gelatin. Due to its catabolic activity this enzyme is involved in bone and cartilage remodeling, and resorption processes including diseases such as osteoporosis, osteolytic bone metastasis, and rheumatoid arthritis, and the loss of lung elasticity and recoil in emphysems. Cathepsin K inhibitors, such as odanacatib, show great potential in the treatment of osteoporosis.[1-5] Cathepsin K is also expressed in a significant fraction of human breast cancers where it could contribute to tumor invasiveness.[6] Cathepsin K expression and its enzyme activity gradually increase in the process of adipocyte differentiation. Moreover, E-64, a Cathepsin K inhibitor, could prevent adipocyte differentiation in a dose-dependent manner. Therefore, it seems that Cathepsin K may be involved in the pathogenesis of obesity by promoting adipocyte differentiation.[7]

The Cathepsin K Activity Assay Kit provides all the reagents required for a highly sensitive detection of human Cathepsin K activity in cell extracts, cell culture media, tissue extracts, body fluids (serum or plasma), and purified enzyme preparations, and for inhibitor screening. The kit was tested with lysates prepared from HFF and SF primary cells, and A549 and MG-63 cell lines. It was tested also with human serum and plasma.

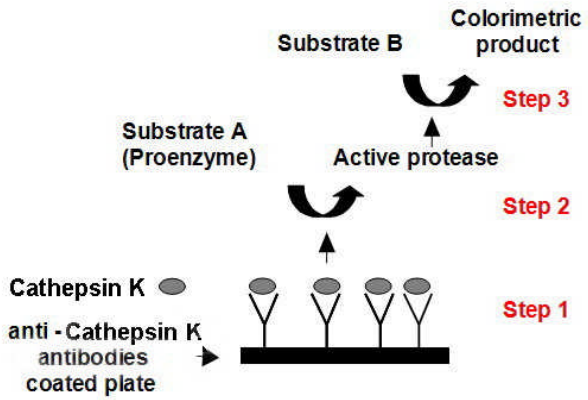
The protease activity measurement is based on a multistep series of reactions (see Figure 1). In the kit assay, steps 2 and 3 are performed simultaneously.[8]

Step 1: The Cathepsin K containing extract is applied into a well, coated with a Cathepsin K specific antibody (supplied with the kit).

Step 2: A modified protein substrate (Substrate A) is added to the well. Substrate A is a proenzyme containing the Cathepsin K protease specific cleavage site fused to another protease. The proenzyme substrate is cleaved by Cathepsin K to form an active "new" protease.

Step 3: A chromogenic peptide substrate (Substrate B) for the "new" protease is added to the well and is cleaved by the "new" protease. The change in the absorption of the chromogenic product is measured at 405 nm. The Cathepsin K activity is directly proportional to the generation of color.

Figure 1.
Principle of Cathepsin K Assay



This assay is sensitive and specific. The enhanced sensitivity is achieved by the signal amplification via the chain reaction. The specificity is achieved by both the immunochemical isolation of Cathepsin K enzyme from the extract by specific antibodies bound to the 96-well plate, and by the use of an enzyme substrate (Substrate A) containing a Cathepsin K specific cleavage site.

Components

The kit is sufficient for 96 reactions in the anti-Cathepsin K coated 96-well plate.

Assay Buffer Catalog Number A9982	20 mL
Wash Buffer Catalog Number W0394	120 mL

Cathepsin K Standard Catalog Number C9119	25 µL
Substrate A (proenzyme) Catalog Number S6323	250 µL
Substrate B Catalog Number S7322	1 mL
Anti-Cathepsin K coated 96-well plate Catalog Number C9244	1 each
DTT, 1 M Catalog Number D7059	400 µL

Equipment Needed but Not Provided
- Plate reader
- Humidified chamber
- Multichannel pipettor

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The anti-Cathepsin K coated 96-well plate (Catalog Number C9244) is composed of twelve 8-well strips. Before use, allow the anti-Cathepsin K coated 96-well plate (the frame with the required number of strips) to warm to room temperature. Unused strips should be stored in a tightly closed nylon bag with a desiccant pack at 2–8 °C.

- Before performing the assay, thaw the Wash buffer (Catalog Number W0394) and Assay Buffer (Catalog Number A9982) at room temperature, and DTT (Catalog Number D7059), Substrate A (Catalog Number S6323), and Substrate B (Catalog Number S7322) on ice. Ensure that the solutions are homogenous by gentle mixing.

DTT Assay Solution – Dilute the DTT (Catalog Number D7059) 10-fold with Assay Buffer before the test (10 µL of the DTT Assay Solution are sufficient for ~30 wells/reactions).

Reaction Mixture – The Reaction Mixture should be freshly prepared before step 4 of the procedure. For 10 wells/reactions prepare 1 mL of Reaction Mixture composed of:
- 875 µL of Assay Buffer (Catalog Number A9982)
- 25 µL of Substrate A (Catalog Number S6323)
- 100 µL of Substrate B (Catalog Number S7322)
- 3 µL of the DTT Assay Solution

For a different number of reactions calculate the volumes required accordingly. Keep the prepared Reaction Mixture on ice until needed for the reaction. Store the remaining Substrate A, Substrate B, and DTT at –20 °C and the Assay Buffer and Wash Buffer at 2–8 °C.

Standard Solutions - Just prior to beginning the assay, dilute an aliquot of the Cathepsin K Standard (160 ng/mL, Catalog Number C9119) in Wash Buffer (Catalog Number W0394) according to Table 1. Mix well after each dilution. Store the Standard Solutions on ice until use. The Standard Solutions will be used to determine a standard curve of Cathepsin K activity.

Table 1.
Serial Dilutions of 160 ng/ml Cathepsin K Standard

Standard sample	Cathepsin K standard (µL)	Wash Buffer (µL)	Cathepsin K Standard final concentration (ng/mL)
1	5 (from 160 ng/mL)	495	1.6
2	250 (from 1.6 ng/mL)	250	0.8
3	250 (from 0.8 ng/mL)	250	0.4
4	250 (from 0.4 ng/mL)	250	0.2
5	250 (from 0.2 ng/mL)	250	0.1
6	250 (from 0.1 ng/mL)	250	0.05
7	250 (from 0.05 ng/mL)	250	0.025
Blank	0	250	0

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C is recommended for all the components **except** for the Anti-Cathepsin K coated 96-well plate, which should be stored at 2–8 °C. Once thawed, the Assay Buffer and Wash Buffer can be stored at 2–8 °C and the Cathepsin K Standard should be stored in aliquots at –70 °C. Avoid repeated freeze-thaw cycles of the Cathepsin K standard.

Procedure

Cathepsin K Activity Assay

When assaying multiple samples, the Cathepsin K coated wells and Substrate A should be from the same lot.

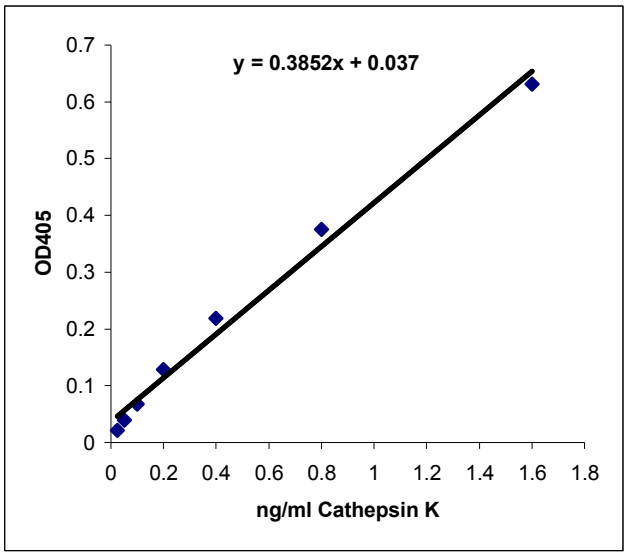
It is recommended to work in duplicates.

1. Pipette 100 µL of each standard and blank (see Table 1) separately into the appropriate well.
2. Pipette 100 µL of the test samples into other wells.
   Note: Samples with high Cathepsin K activity should be diluted with Wash Buffer.
3. Cover the plate with the lid and incubate for one hour at room temperature.
4. Aspirate the solution from the wells and wash the wells 4 times with 200 µL each of Wash Buffer.
5. Blot the plate on tissue paper to remove any residual solution.
6. Pipette 100 µL of Reaction Mixture into each well. Cover the plate with the lid and incubate for 1–2 hours at 37 °C in a humidified chamber (i.e., a closed box with a source for humidity such as wet paper).
   Note: The incubation duration depends on the Cathepsin K concentration and activity in the sample. For lower levels of Cathepsin K, longer incubation periods (up to 20 hours) may be required.
7. Remove the lid and measure the absorbance at 405 nm using a plate reader.
8. Calculate the sample activity using a standard curve.

Calculations

1. Calculate the average absorbance (of the duplicates) of the blank, each standard concentration, and the test sample. Subtract the average blank value from the average value of each standard and sample.
2. Plot the average absorbance of each standard concentration (y-axis) as a function of the Cathepsin K concentration in the well (x-axis).

Figure 2.
Typical Standard Curve for Cathepsin K (0.025–1.6 ng/mL)



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United States Patent 5,811,252
European Patent 0,691,409B1
Japanese Patent Application 8,056,665A2

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