

NAMPT Activity Assay Kit (Colorimetric) ab221819

3 References [画像数 6](#)

製品の概要

製品名	NAMPT Activity Assay Kit (Colorimetric)
検出方法	Colorimetric
サンプルの種類	Cell Lysate, Purified protein
アッセイタイプ	Semi-quantitative
種交差性	交差種: Mammals
製品の概要	NAMPT Activity Assay Kit (Colorimetric) (ab221819) provides a sensitive and robust method to evaluate activators and inhibitors of nicotinamide phosphoribosyltransferase (NAMPT) activity using recombinant NAMPT protein. The assay can also be used to measure activity from endogenous NAMPT immunoprecipitated from cell extracts. The assay is based on a multi-step reaction that converts WST-1 to WST-1 formazan, which can be easily detected at OD 450 nm. As the reaction is not stopped, it is necessary to monitor the absorbance increase of WST-1 formazan at OD450 nm at regular intervals after the reaction is initiated to determine velocity of reaction.

Detection of NAMPT activity can be measured with a One-Step or a Two-Step method. For the One-Step method, the four enzymes involved in the reaction are mixed together. The detection sensitivity of this method is lower than that of the Two-Step method since three coupled reactions occur simultaneously. We recommend performing the One-step assay only when using purified protein. The Two-Step method is performed by adding the first two enzymes to produce NAD^+ , followed by the addition of the rest of the components to form WST-1 formazan by NAD/NADH enzyme cycling reaction. We recommend performing the Two-Step assay if using immunoprecipitated cell lysates, as the assay procedure allows to check for contamination and interference for NAD^+ present in the sample. Since the assay is based on a NAD^+ detection system, it is not possible to directly detect NAMPT activity in crude cell extracts, which contain relatively high NAD^+ .

For the One-Step method, we describe a procedure to test NAMPT activity using purified enzyme and a separate procedure for screening NAMPT activity inhibitors. For the Tw-Step Method, we describe two different procedures to test NAMPT activity using purified enzyme or to test immunoprecipitated cell lysates. There is also a separate procedure for screening NAMPT activity inhibitors.

特記事項

Nicotinamide phosphoribosyltransferase (NAMPT, visfatin, pre-B-cell colony-enhancing factor, EC 2.4.2.12), is the rate-limiting enzyme that converts nicotinamide to nicotinamide mononucleotide (NMN) in the NAD^+ salvage pathway of NAD^+ biosynthesis in mammals. The

expression of NAMPT is upregulated during activation of immune cells such as monocytes, macrophages, dendritic cells, T and B cells, as well as in amniotic epithelial cells upon stimulation with several inflammatory cytokines. The NAMPT-specific inhibitor FK866 has been found to deplete intracellular NAD content and result in apoptotic cell death in many cancer cell lines without any DNA damage.

試験プラットフォーム

Microplate reader

製品の特性

保存方法

Store at -80°C. Please refer to protocols.

内容	100 tests
ADH	1 x 500µl
ATP	1 x 500µl
Diaphorase	1 x 500µl
EtOH Solution	1 x 500µl
NAMPT	1 x 500µl
NAMPT Assay Buffer (20X)	1 x 1ml
Nicotinamide	1 x 500µl
NMNAT1	1 x 500µl
PRPP	1 x 500µl
WST-1	1 x 500µl

機能

Catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, an intermediate in the biosynthesis of NAD. It is the rate limiting component in the mammalian NAD biosynthesis pathway.

組織特異性

Expressed in large amounts in bone marrow, liver tissue, and muscle. Also present in heart, placenta, lung, and kidney tissues.

パスウェイ

Cofactor biosynthesis; NAD(+) biosynthesis; nicotinamide D-ribonucleotide from 5-phospho-alpha-D-ribose 1-diphosphate and nicotinamide: step 1/1.

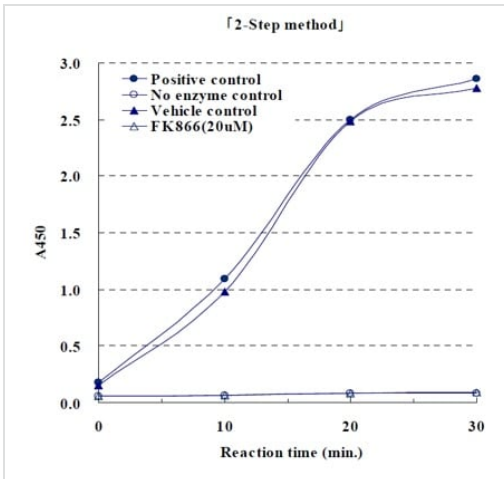
配列類似性

Belongs to the NAPRTase family.

細胞内局在

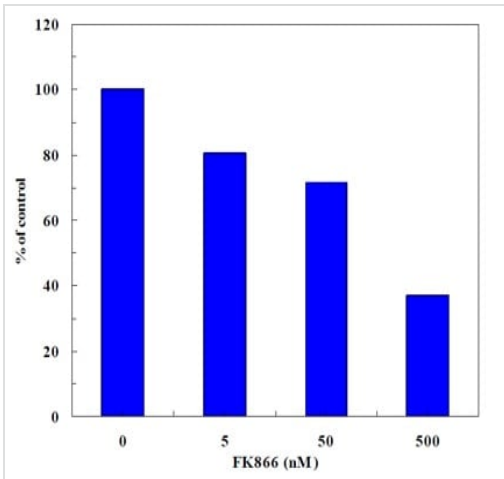
Cytoplasm.

画像



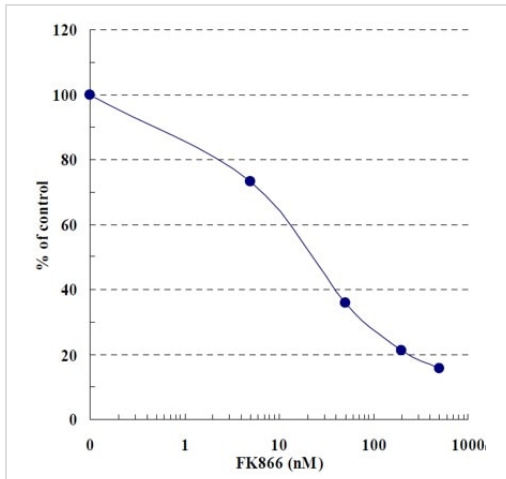
NAMPT Activity Assay Kit (Colorimetric) (ab221819)
Time course kinetic curves for FK866 inhibitory effect

NAMPT Activity Assay Kit (Colorimetric) (ab221819) Time course kinetic curves for FK866 inhibitory effect using Two-Step Assay Method. FK866 effect was tested at 20 μ M (open triangles). Appropriate control reactions [positive control (closed circles), no enzyme control (open circles) and vehicle control (closed triangles)] were run in parallel.



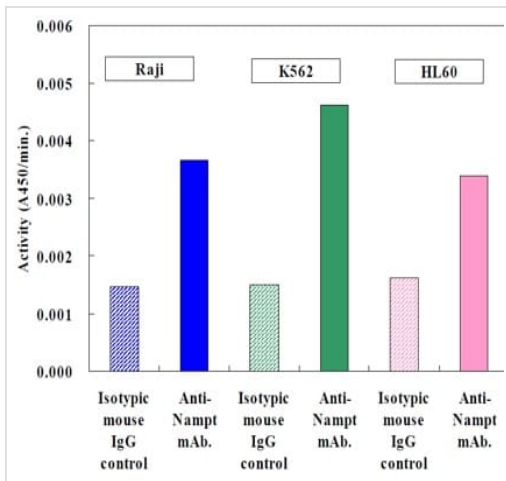
NAMPT Activity Assay Kit (Colorimetric) (ab221819)
Inhibitory effect of increasing concentrations of FK866 on NAMPT

NAMPT Activity Assay Kit (Colorimetric) (ab221819) Inhibitory effect of increasing concentrations of FK866 on NAMPT activity from immunoprecipitated Raji cell lysates.



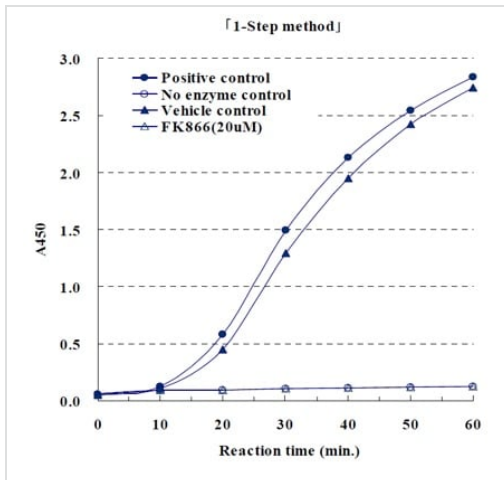
NAMPT Activity Assay Kit (Colorimetric) (ab221819) Inhibitory effect of FK866 on recombinant NAMPT activity using One-Step Assay Method

NAMPT Activity Assay Kit (Colorimetric) (ab221819)
Inhibitory effect of FK866 on recombinant NAMPT activity



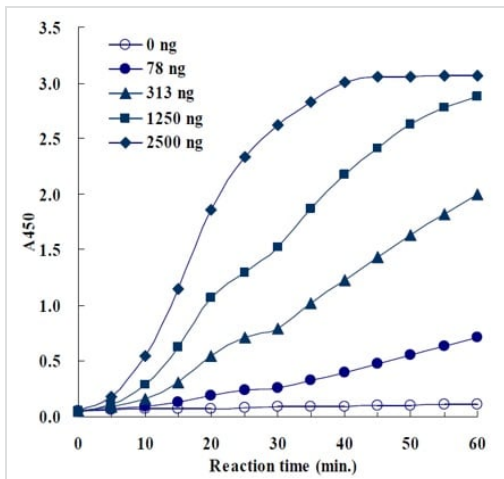
NAMPT Activity Assay Kit (Colorimetric) (ab221819) Measurement of NAMPT activity on immunoprecipitated cell lysates from Raji, K562 and HL60 cell extracts. Immunoprecipitate from isotypic mouse IgG control shows background activity compared to anti-NAMPT immunoprecipitate.

NAMPT Activity Assay Kit (Colorimetric) (ab221819)
Measurement of NAMPT activity



NAMPT Activity Assay Kit (Colorimetric)
(ab221819) Time course kinetic curves for FK866

NAMPT Activity Assay Kit (Colorimetric) (ab221819) Time course kinetic curves for FK866 inhibitory effect using One-Step Assay Method. FK866 effect was tested at 20 μ M (open triangles). Appropriate control reactions [positive control (closed circles), no enzyme control (open circles) and vehicle control (closed triangles)] were run in parallel.



NAMPT Activity Assay Kit (Colorimetric) (ab221819)
Time course kinetic curve

NAMPT Activity Assay Kit (Colorimetric) (ab221819) Time course kinetic curve of recombinant NAMPT activity using One-Step Assay Method

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