abcam

Product datasheet

P4HB Inhibitor Screening Assay Kit ab139480

2 References 3 图像

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MIT.	7/1
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产品名称 P4HB抑制剂Screening Assay试剂盒

检测方法 Fluorescent

样品类型 Purified protein, Inhibitor compounds

检测类型 Enzyme activity

灵敏度 37 μM

产品概述 Abcam's P4HB (PDIA1) Inhibitor Screening Assay Kit (ab139480) provides a simple,

homogenous assay for screening modulators of protein disulfide isomerase (PDIA1) enzymatic activity in a 96-well microplate. The basis for the assay is the P4HB (PDIA1)-catalyzed reduction of insulin in the presence of DTT, resulting in the formation of insulin aggregates which in turnn

bind to the red-emitting fluorogenic P4HB (PDIA1) Detection Reagent.

Relative to analogous turbidimetric assays of P4HB (PDIA1) activity, the fluorescence-based assay provides a vastly improved assay signal window, improved lower detection limit and $\frac{1}{2}$

superior Z'-score (>0.8).

The kit includes human recombinant P4HB (PDIA1) enzyme as positive control, as well as the

P4HB (PDIA1) inhibitor bacitracin as a negative control.

This kit will provide a quantitative readout of P4HB (PDIA1) enzymatic activity is a robust and HTP fashion and can be applied to identification of P4HB (PDIA1) inhibitors from chemical libraries.

平台 Microplate reader

性能

存放说明 Please refer to protocols.

Deionized Water 1 x 5ml DTT 2 x 1.3ml	组 件	2 x 96 tests
DTT 2 x 1.3ml	Deionized Water	1 x 5ml
	DTT	2 x 1.3ml

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组 件	2 x 96 tests
Inhibitor Control (Bacitracin)	1 vial
Insulin (from bovine pancreas)	2 vials
PBE Buffer	1 x 25ml
P4HB (PDIA1) (Human, Recombinant)	2 x 165µl
P4HB (PDIA1) Detection Reagent	1 x 20µl
Stop Reagent	1 x 1ml

功能

This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP.

序列相似件

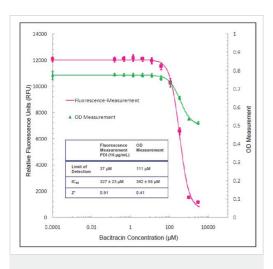
Belongs to the protein disulfide isomerase family.

Contains 2 thioredoxin domains.

细胞定位

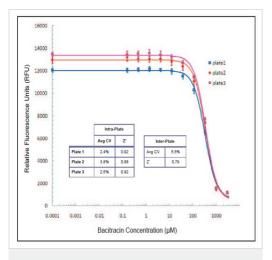
Endoplasmic reticulum lumen. Melanosome. Cell membrane. Highly abundant. In some cell types, seems to be also secreted or associated with the plasma membrane, where it undergoes constant shedding and replacement from intracellular sources (Probable). Localizes near CD4-enriched regions on lymphoid cell surfaces. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

图片

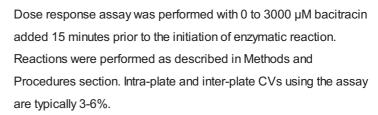


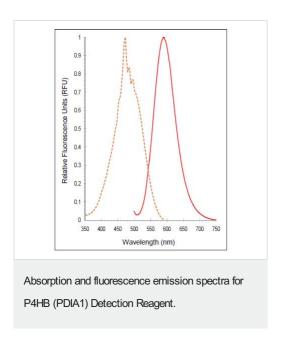
Assay validation using Inhibitor Control (bacitracin).

Dose response assay was performed with 0 to 3000 μ M bacitracin added 15 min prior to the initiation of enzymatic reaction. Reactions were performed as described in Assay Protocol section. The fluorescence-based assay provides a vastly improved assay signal window and improved lower detection limit, In addition, the Z'-factor score obtained using the assay (0.91 for assay with and without P4HB) demonstrates excellent signal-to-noise and signal-to-background ratio



Intra-plate and inter-plate reproducibility using Inhibitor Control (bacitracin)





All spectra were determined in PBE Buffer. Example data only.

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