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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet
MMP3 (K45E) Inhibitor Screening Assay Kit
Catalog #79907
Size: 384 reactions

BACKGRUND: Matrix metalloproteinase 3 (MMP3) is a zinc-dependent endopeptidase involved in the breakdown of extracellular matrix and tissue remodeling in normal physiological processes, such as embryonic development and reproduction, as well as in disease processes, such as arthritis and cancer. Overexpression of MMPs is associated with tumor invasion, neoangiogenesis and metastasis. Several inhibitors of MMPs (MMPI), small molecules and blocking monoclonal antibodies, have entered clinical trials. MMP3 (K45E) variant is associated with differences in MMP3 activity and has been linked to cancer susceptibility.

DESCRIPTION: The *MMP3 (K45E) Inhibitor Screening Assay Kit* is designed to measure the endopeptidase activity of MMP3 (K45E) for screening and profiling applications. The MMP3 (K45E) assay kit comes in a convenient 384-well format, with purified MMP3 (K45E), its fluorogenic substrate, and MMP buffer for 384 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
11346	MMP3 (K45E)	50 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	AMPA (100 mM)	10 µl	-20 °C	
	MMP3 substrate (6 mM)	20 µl	-20 °C	
	4X MMP3 buffer	10 ml	-20 °C	
	384-well black microplate	1	Room Temp	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Adjustable micropipettor and sterile tips
Fluorescent microplate reader

APPLICATIONS: Great for studying enzyme kinetics and screening small molecule inhibitors and antibodies for drug discovery and HTS applications.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES:
Nagase, H., *et al. Biochemistry.* 1990. **29(24):** 5783-5789.
Mendes, O., *et al. Clin Exp Metastasis.* 2005. **22(3):** 237-246.
Dormán, *et al. Drugs.* 2010. **70(8):** 949-964.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1. Prepare **1x MMP buffer** by diluting **4x MMP buffer** 4-fold with distilled water. For example, prepare 10 ml **1x MMP3 buffer** by mixing 2.5 ml **4x MMP3 buffer** with 7.5 ml water. Dilute only enough buffer required for the assay. Store remaining **4x MMP buffer** at -20°C in single-use aliquots.
2. Thaw **MMP3 (K45E)** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Prepare **activated MMP3 (K45E)** by diluting the enzyme to 100 ng/µl in **1x MMP3 buffer** containing 1 mM AMPA for 1 hour at 37ºC. Aliquot remaining **MMP3 (K45E)** enzyme into single-use aliquots at -80°C. Note: **MMP3 (K45E)** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
3. Prepare **Enzyme solution** (5 ng/µl MMP3 (K45E)) by diluting 100 ng/µl **activated MMP3 (K45E)** 20-fold in **1x MMP3 buffer**. For example, to prepare 1000 µl, add 50 µl of 100 ng/µl **activated MMP3 (K45E)** to 950 µl of **1X MMP3 buffer**.
4. Add 20 µl of **Enzyme solution** (5 ng/µl MMP3 (K45E)) to each well designated "Positive Control", and "Test Inhibitor" and 20 µl of **1x MMP3 buffer** to each well designated "Blank".
5. Add 5 µl of **Test Inhibitor** solution to each well designated "Test Inhibitor". For the wells labeled "Positive Control" and "Blank", add 5 µl of **Inhibitor buffer** (same solution without inhibitor).
6. Prepare **Substrate solution** (10 µM) by diluting **MMP3 substrate (6 mM)** 600-fold in **1x MMP3 buffer**. For example, to prepare 1 ml **Substrate solution**, add 1.67 µl of **MMP3 substrate (6 mM)** to 998.3 µl of **1x MMP3 buffer**.
7. Add 25 µl of **Substrate solution** (10 µM) to all wells. Incubate reaction at room temperature and protected from light for 30 minutes.
8. Read fluorescence intensity of the samples ($\lambda_{excitation}$ = 320 nm; $\lambda_{emission}$ = 405 nm) in an appropriate microplate reader.

	Positive Control	**Test Inhibitor**	**Blank**
Enzyme solution (5 ng/µl MMP3 (K45E))	20 µl	20 µl	-
1x MMP3 buffer	-	-	20 µl
Test inhibitor	-	5 µl	-
Inhibitor buffer (no inhibitor)	5 µl	-	5 µl
Substrate solution (10 µM)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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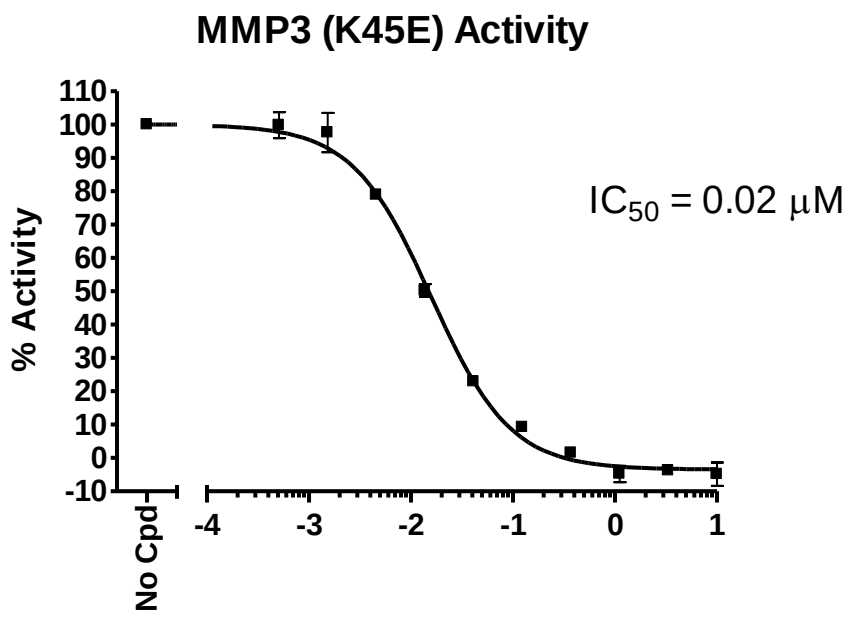
Example of assay results:



MMP3 (K45E) inhibition by N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH, Sigma #SML0584) measured using the *MMP3 (K45E) Inhibitor Screening Assay Kit*, BPS Bioscience Catalog #79907. Fluorescence was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:

Product	**Catalog#**	**Size**
MMP1	80214	20 µg
MMP2	80213	20 µg
MMP3 (K45E)	11346	50 µg
MMP8	100552	100 µg
MMP9 (Q279R)	80215	20 µg