

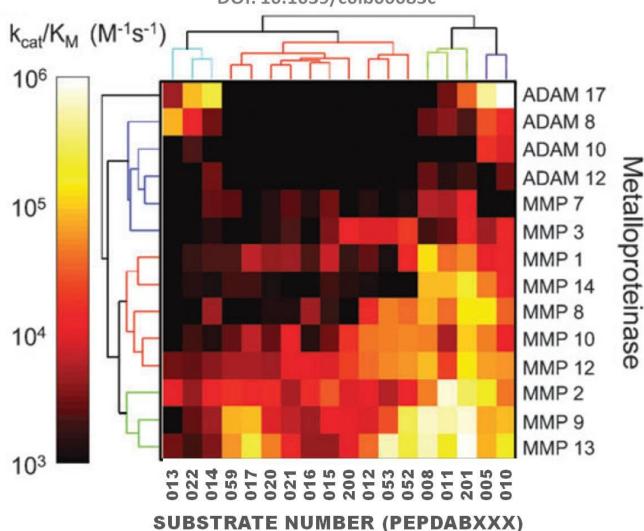
BIOZYME, INC.

PRODUCT CATALOG 2015

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Chart from Integrative Biology, 2011, 3, 422-438, Miller, M. A., et al., DOI: 10.1039/c0ib00083c





NEW PROMOTIONS

BioZyme, Inc now offers a new substrate, PEPMCA002, or MCA-LAQAHomopheRSK(Dnp)NH2 that is comparable to PEPDAB005 in terms of its activity against ADAM and MMP family members. However, it contains MCA and Dnp as the fluorophore pair instead of Dabcyl/FAM. We have a new promotional price of \$80 for 1mg.

BioZyme, Inc is also the only company to offer selective substrates for ADAM10 and ADAM17. We currently have 3 new products, PEPMCA001, an ADAM10 selective substrate that has MCA/Dnp as the fluorophore pair, PEPDAB063, an ADAM10 selective substrate that has Dabcyl/FAM as the fluorophore pair, and PEPDAB064, the most sensitive substrate for ADAM17 known to date.

Please visit our Website at www.biozyme-inc.com for online ordering.

For technical support, contact FHRasmussen@biozyme-inc.com



PRODUCT LIST

ORDER ONLINE

CATALOG	DESCRIPTION	AMOUNT	PRICE
NUMBER			(USD)
PEPMCA002m001	ADAM17,12,10,8, and MMP Substrate	1mg	80
PEPDAB005m001	ADAM17,12,10,8, and MMP Substrate	1 mg	125
PEPDAB005m005	ADAM17,12,10,8, and MMP Substrate	5 mg	500
PEPDAB008m001	MMP1,2,3,8,9,12,13, and 14 Substrate	1 mg	125
PEPDAB008m005	MMP1,2,3,8,9,12,13, and 14 Substrate	5 mg	500
PEPDAB010m001	ADAM17,10,9 and MMP Substrate	1 mg	125
PEPDAB010m005	ADAM17,10,9 and MMP Substrate	5 mg	500
PEPDAB011m001	MMP13 Specific Substrate	1 mg	125
PEPDAB011m005	MMP13 Specific Substrate	5 mg	500
PEPDAB013m001	ADAM8 Specific Substrate	1 mg	125
PEPDAB013m005	ADAM8 Specific Substrate	5 mg	500
PEPDAB014m001	ADAM17 Specific Substrate	1 mg	125
PEPDAB014m005	ADAM17 Specific Substrate	5 mg	500
PEPDAB015m001	General ADAM Substrate	1 mg	125
PEPDAB015m005	General ADAM Substrate	5 mg	500
PEPDAB016m001	Specificity: MMP2 /MMP9Substrate	1 mg	125
PEPDAB016m005	Specificity: MMP2 /MMP9Substrate	5 mg	500
PEPDAB017m001	Specificity: MMP9/MMP2 Substrate	1 mg	125
PEPDAB017m005	Specificity: MMP9/MMP2 Substrate	5 mg	500
PEPDAB022m001	General ADAM Substrate	1 mg	125
PEPDAB022m005	General ADAM Substrate	5 mg	500
PEPDAB052m001	MMP13 Selective; Specificity for	1 mg	125
	MMP9 over MMP2 Substrate		
PEPDAB052m005	MMP13 Selective; Specificity for	5 mg	500
	MMP9 over MMP2 Substrate		
PEPDAB053m001	MMP13 Selective; Specificity for	1 mg	125
	MMP9 over MMP2 Substrate		
PEPDAB053m005	MMP13 Selective; Specificity for	5 mg	500
	MMP9 over MMP2 Substrate		
PEPMCAm001	ADAM10 Sensitive and Selective	1 mg	150
PEPMCAm005	ADAM10 Sensitive and Selective	5 mg	500
PEPDAB061m001	MMP3 and MMP1 Substrate; General	1 mg	150
	MMP substrate		
PEPDAB061m005	MMP3 and MMP1 Substrate; General	5 mg	500
	MMP substrate		

PEPDAB063m001	ADAM10 Selective and Sensitive;	1 mg	150
	Best for cell based assay		
PEPDAB063m005	ADAM10 Selective and Sensitive;	5 mg	500
	Best for cell based assay		
PEPDAB064m001	Highly sensitive for ADAM17	1 mg	150
PEPDAB064m005	Highly sensitive for ADAM17	5 mg	500
ENZHADAM8u010	Recombinant Human ADAM8	10 ug	150
ENZHADAM8u020	Recombinant Human ADAM8	20 ug	275



ADAM AND MMP SUBSTRATE

Fluorescent Substrate: MCA-LAQAPhe(homo)RSK(Dnp)-NH₂

Catalog Number: PEPMCA002

Use: This fluorescent peptide substrate can be used to assess activity of enzymes in

the ADAM and MMP family. It is the MCA/Dnp version of PEPDAB005. Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10 μ M concentration. For use with ADAM10 and 17, the buffer should consist of 25mM Tris, pH 8, and 6 x 10⁻⁴ Brij detergent. If human ADAM8 or ADAM12 are used, add enough CaCl₂ to the aforementioned buffer to achieve a concentration of 10mM. For use with the MMPs, the buffer should contain 50 mM

Tris, pH 7.5, 150 mM NaCl, 2 mM CaCl $_2$, 5 μ M ZnSO $_4$, and 0.01% Brij-35. Excitation and emission wavelengths are 325 and 393 nm respectively.

Molecular Weight: 1315.4 g/mol

Purity: Greater than 92% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10% Formic acid

Appearance: Yellow lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.

Stability: Samples are stable up to 6 months at -70°C.

Reference: Fluorescent substrates for the proteinases ADAM17, ADAM10, ADAM8, and ADAM12 useful

for high-throughput inhibitor screening, Moss, M.L. and Rasmussen, F.H. (2007) Analytical

Biochemistry; 366(2):144-8.



Fluorescent Substrate: Dabcyl-LAQAPhe(homo)RSK(5FAM)-NH₂

Catalog Number: PEPDAB005

Use: This fluorescent peptide substrate can be used to assess activity of enzymes in

the ADAM and MMP families. It demonstrates reasonably strong activity against all of those enzymes, with specificity constants, k_{cat}/K_m ($M^{-1}s^{-1}$), ranging from approximately 4 x 10³ to 4 x 10⁵ (see Table 1 below, column highlighted in blue).

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM10 and 17, the buffer should consist of 25mM Tris, pH 8,

10mM CaCl₂, and 6 x 10⁻⁴ Brij detergent

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1542.5 g/mol

Purity: Greater than 92% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10% Formic acid

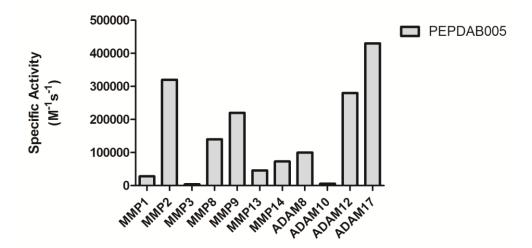
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity constants, k_{cat}/K_m ($M^{-1}s^{-1}$), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB005)	
MMP1	2.8 x 10 ⁴	
MMP2	3.2 x 10 ⁵	
MMP3	4.0×10^3	
MMP8	1.4 x 10 ⁵	
MMP9	2.2 x 10 ⁵	
MMP13	4.6 x 10 ⁵	
MMP14	7.3×10^4	
ADAM8	1.0 x 10 ⁵	
ADAM10	6.2 x 10 ³	
ADAM12	2.8×10^5	
ADAM17 (TACE)	4.3×10^5	

^a ND, no turnover detected

References:

- 1. Fluorescent substrates for the proteinases ADAM17, ADAM10, ADAM8, and ADAM12 useful for high-throughput inhibitor screening. Marcia L Moss, Fred H Rasmussen. (2007) Analytical Biochemistry; 366(2):144-8. PMID 17548045
- 2. Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.
- 3. Potential of Fluorescent Metalloproteinase Substrates for Cancer Detection. Roopali Roy, 1,5

 David Zurakowski, 2,5

 Susan Pories, Marcia L. Moss, 4,* and Marsha A. Moses 1,5

 Clin Biochem. 2011

 December; 44(17-18): 1434–1439.

^b NA, not attempted



Fluorescent Substrate: Dabcyl-PChaGC(Me)HAK(5FAM)-NH₂

Catalog Number: PEPDAB008

Use: This fluorescent peptide substrate can be used to assess activity of enzymes in

the ADAM and MMP families. It demonstrates reasonably strong activity against all of those enzymes, with specificity constants, k_{cat}/K_m (M⁻¹s⁻¹), ranging from approximately 10¹ to 10⁶ (see Table 1 below, column highlighted in red).

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM10 and 17, the buffer should consist of 25mM Tris, pH 8, and 6 x 10⁻⁴ Brij detergent. If human ADAM8 or ADAM12 are used, add enough CaCl₂ to the aforementioned buffer to achieve a concentration of 10mM.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl $_2$, 5 μ M ZnSO $_4$, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1388.3 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10% Formic acid

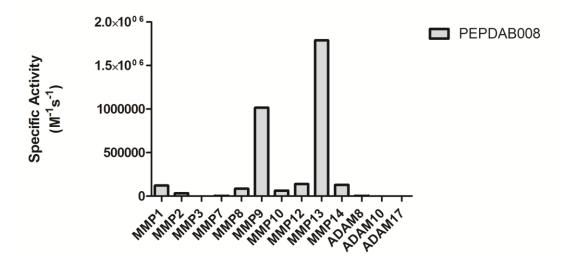
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity constants, k_{cat}/K_m ($M^{-1}s^{-1}$), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB008)
MMP1	7.6 x 10 ⁴
MMP2	2.9 x 10 ⁴
MMP3	5.2 x 10 ¹
MMP8	NA
MMP9	8.5 x 10 ⁵
MMP13	2.1 x 10 ⁶
MMP14	1.9 x 10 ³
ADAM8	2.6 x 10 ³
ADAM10	5.6 x 10 ¹
ADAM12	3.0 x 10 ³
ADAM17 (TACE)	6.8 x 10 ³

^a ND, no turnover detected

References:

- 1. Fluorescent substrates for the proteinases ADAM17, ADAM10, ADAM8, and ADAM12 useful for high-throughput inhibitor screening. Marcia L Moss, Fred H Rasmussen. (2007) Analytical Biochemistry; 366(2):144-8. PMID 17548045
- 2. Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.
- 3. <u>Potential of Fluorescent Metalloproteinase Substrates for Cancer Detection.Roopali Roy</u>, ^{1,5} <u>David Zurakowski</u>, ^{2,5} <u>Susan Pories</u>, ³ <u>Marcia L. Moss</u>, ^{4,*} and <u>Marsha A. Moses</u>, ^{1,5} <u>Clin Biochem. 2011</u> <u>December</u>; 44(17-18): 1434–1439.

^b NA, not attempted



Fluorescent Substrate: Dabcyl-SPLAQAVRSSK(5FAM)-NH₂

Catalog Number: PEPDAB010

Use: This fluorescent peptide substrate can be used to assess activity of enzymes in

the ADAM family. It demonstrates reasonably strong activity against ADAM17,

ADAM9, and ADAM10.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM10, 9 and 17, the buffer should consist of 25mM Tris, pH 8,

and 6 x 10⁻⁴ Brij detergent.

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1751.9 g/mol

Purity: Greater than 93% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10% Formic acid

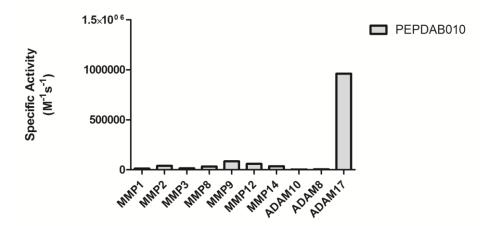
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity values, (M⁻¹s⁻¹), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB010)
MMP1	5.1 x 10 ⁴
MMP2	1.7 x 10 ⁵
MMP3	3.9×10^4
MMP8	2.6×10^4
MMP9	6.0×10^5
MMP13	1.7 x 10 ⁶
MMP14	NA
ADAM8	3.3 x 10 ⁴
ADAM9	1.1 x 10 ⁵
ADAM10	3.6×10^3
ADAM12	3.8×10^4
ADAM17 (TACE)	9.6×10^5
3	

^a ND, no turnover detected

References:

- 1. Fluorescent substrates useful as high-throughput screening tools for ADAM9.Marcia L Moss, Fred H Rasmussen, Raphael Nudelman, Peter J Dempsey, Jason Williams Combinatorial chemistry & high throughput screening 12/2009; 13(4):358-65.
- 2. <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities.</u> <u>Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.</u>
- 3. <u>High-throughput protease activity cytometry reveals dose-dependent heterogeneity in PMA-mediated ADAM17 activation.</u> Wu L, et al. Integr Biol (Camb). 2015 May;7(5):513-24. doi: 10.1039/c5ib00019j. Epub 2015 Apr 2. BACK TO PRODUCT LIST

^b NA, not attempted



Fluorescent Substrate: Dabcyl-GPLGMRGK(5FAM)-NH₂

Catalog Number: PEPDAB011

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs. It

provides good selectivity since it is not processed very well by the ADAMs tested. Its specificity constant, k_{cat}/K_m ($M^{-1}s^{-1}$), is 4.3 x 10⁶ and 1.4 x 10⁶ respectively,

against MMP13 and MMP9.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl $_2$, 5 μ M ZnSO $_4$, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

For specific information, see reference:

Molecular Weight: 1423.4 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

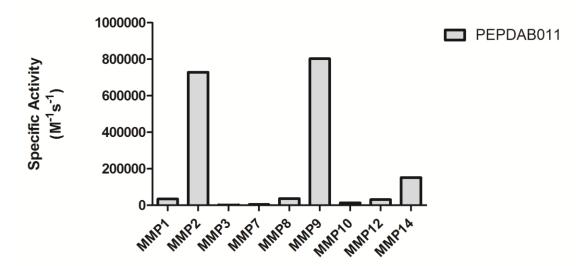
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Reference:

1.<u>Use of a multiple-enzyme/multiple-reagent assay system to quantify activity levels in samples containing mixtures of matrix metalloproteinases.Fred H Rasmussen, Nolan Yeung, Laura Kiefer, Gillian Murphy, Carlos Lopez-Otin, Michael P Vitek, Marcia L Moss . 04/2004; 43(11):2987-95.</u>

2. Potential of Fluorescent Metalloproteinase Substrates for Cancer Detection. Roopali Roy, 1,5 David Zurakowski, 2,5 Susan Pories, Marcia L. Moss, 4,7 and Marsha A. Moses 1,5 Clin Biochem. 2011 December; 44(17-18): 1434–1439.



Fluorescent Substrate: Dabcyl-HGDQMAQKSK(5FAM)-NH₂

Catalog Number: PEPDAB013

Use: This fluorescent peptide substrate is used primarily to assess activity of ADAM8,

though it is also processed to a small extent by MMP2, ADAM10, and ADAM12. It provides good selectivity since it is not processed at all by the seven other MMPs and ADAMs tested. Its specificity constant, k_{cat}/K_m ($M^{-1}s^{-1}$), is 5.3 x 10^4 against ADAM8, and 2.4 x 10^3 , 2.7 x 10^2 , and 4.0 x 10^1 against MMP2, ADAM10, and ADAM12 respectively (see Table 1 below, column highlighted in blue).

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM8, the buffer should consist of 25mM Tris, pH 8, 6 x 10^{-4} Brij detergent, and 10mM CaCl₂. If used with ADAM10, the CaCl₂ is not required.

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1737.7 g/mol

Purity: Greater than 94% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

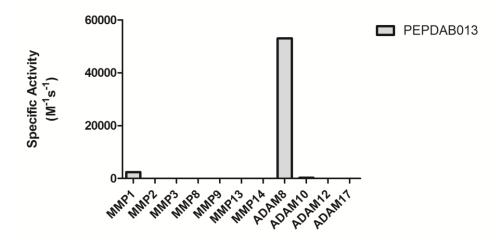
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity constants, k_{cat}/K_m ($M^{-1}s^{-1}$), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB013)
MMP1	ND
MMP2	2.4 x 10 ³
MMP3	ND
MMP8	ND
MMP9	ND
MMP13	ND
MMP14	ND
ADAM8	5.3 x 10 ⁴
ADAM10	2.7×10^2
ADAM12	4.0 x 10 ¹
ADAM17 (TACE)	ND

^a ND, no turnover detected

References:

- 1. Fluorescent substrates for the proteinases ADAM17, ADAM10, ADAM8, and ADAM12 useful for high-throughput inhibitor screening. Marcia L Moss, Fred H Rasmussen. (2007) Analytical Biochemistry; 366(2):144-8. PMID 17548045
- 2. <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.</u>
- 3. <u>Potential of Fluorescent Metalloproteinase Substrates for Cancer Detection. Roopali Roy</u>, ^{1,5} <u>David Zurakowski</u>, ^{2,5} <u>Susan Pories</u>, ³ <u>Marcia L. Moss</u>, ^{4,*} and <u>Marsha A. Moses</u>, ^{1,5} <u>Clin Biochem. 2011</u> December; 44(17-18): 1434–1439.

^b NA, not attempted



Fluorescent Substrate: Dabcyl-EHADLLAVVAK(5FAM)-NH₂

Catalog Number: PEPDAB014

Use: This fluorescent peptide substrate is used primarily to assess activity of ADAM

and MMP families.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10^{-4} Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl $_2$, 5 μ M ZnSO $_4$, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1845.0 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

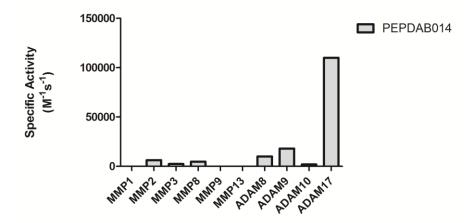
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity values, (M⁻¹s⁻¹), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB014)
MMP1	ND
MMP2	6.3 x 10 ³
MMP3	2.4×10^3
MMP8	4.8×10^3
MMP9	ND
MMP13	ND
MMP14	NA
ADAM8	1.0 x 10⁴
ADAM9	1.8×10^4
ADAM10	1.9 x 10 ³
ADAM12	1.0 x 10 ⁴
ADAM17 (TACE)	1.1 x 10 ⁵

^a ND, no turnover detected

References:

- 1. Fluorescent substrates useful as high-throughput screening tools for ADAM9. Marcia L Moss, Fred H Rasmussen, Raphael Nudelman, Peter J Dempsey, Jason Williams. Combinatorial chemistry & high throughput screening . 12/2009; 13(4):358-65. DOI:10.2174/138620710791054259
- 2. <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.</u>
- 3. Potential of Fluorescent Metalloproteinase Substrates for Cancer Detection. Roopali Roy, 1,5 David Zurakowski, 2,5 Susan Pories, Marcia L. Moss, 4,7 and Marsha A. Moses 1,5 Clin Biochem. 2011 December; 44(17-18): 1434–1439.

^b NA, not attempted



Fluorescent Substrate: Dabcyl-VDLFYLQQPK(5FAM)-NH₂

Catalog Number: PEPDAB015

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10^{-4} Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1858.9 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.

Stability: Samples are stable up to 6 months at -70°C.

References:

1. <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger Integrative Biology 12/2010; 3(4):422-38.</u>



Fluorescent Substrate: Dabcyl-SNLAYYTAK(5FAM)-NH₂

Catalog Number: PEPDAB016

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs

and is most active with MMP13. Selective for MMP2 over MMP9.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10⁻⁴ Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1638.5 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

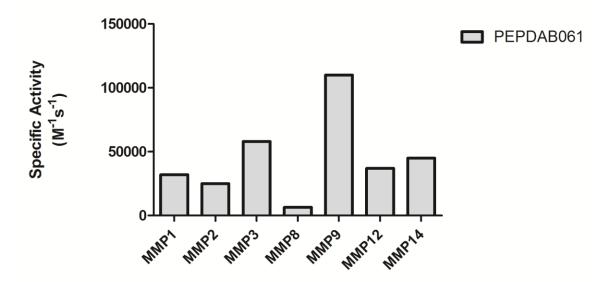
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Reference: <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger.</u> Integrative Biology 12/2010; 3(4):422-38.



Fluorescent Substrate: Dabcyl-APRWIQDK(5FAM)-NH₂

Catalog Number: PEPDAB017

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs

and is most active with MMP13. Selective for MMP9 over MMP2.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below to avoid DMSO effects on the reaction, and

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10⁻⁴ Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1621.6 g/mol

Purity: Greater than 94% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

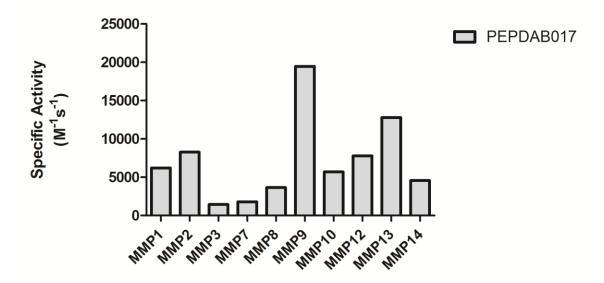
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



References: <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger. Integrative Biology 2010; 3(4):422-38.</u>



Fluorescent Substrate: Dabcyl-LRAEQQRLKSK(5FAM)-NH₂

Catalog Number: PEPDAB022

Use: This fluorescent peptide substrate is used primarily to assess activity of ADAM

proteinases.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about 10μ M concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10^{-4} Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1965 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

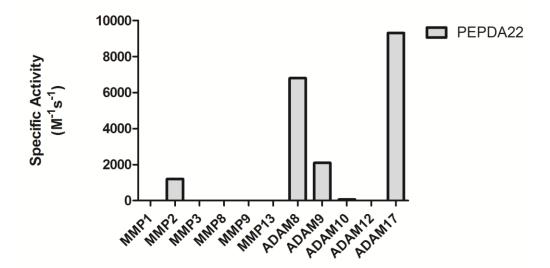
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



Specificity values, (M⁻¹s⁻¹), of substrates tested against MMPs 1, 2, 3, 8, 9, 13, and 14 and ADAMs 8, 10, 12, and 17 (TACE)

Enzyme \ Substrate	(PEPDAB015)
MMP1	ND
MMP2	1.2 x 10 ³
MMP3	ND
MMP8	ND
MMP9	ND
MMP13	ND
MMP14	NA
ADAM8	6.8 x 10 ³
ADAM9	2.1 x 10 ³
ADAM10	6.9 x 10 ¹
ADAM12	ND
ADAM17 (TACE)	9.4 x 10 ³

^a ND, no turnover detected

References:

- 1. <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger. Integrative Biology 2010; 3(4):422-38.</u>
- 2.Fluorescent substrates useful as high-throughput screening tools for ADAM9. Marcia L Moss, Fred H Rasmussen, Raphael Nudelman, Peter J Dempsey, Jason Williams Combinatorial chemistry & high throughput screening 12/2009; 13(4):358-65.

^b NA, not attempted\



Fluorescent Substrate: Dabcyl-APFEMSAK(FAM)-NH₂

Catalog Number: PEPDAB052

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs. It

provides good selectivity since it is not processed very well by the ADAMs tested. Its specificity constant. It is selective for MMP13. It has some specificity of MMP9

over MMP2.

The peptide is already dissolved in DMSO to make a stock solution of about 5mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: NA

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

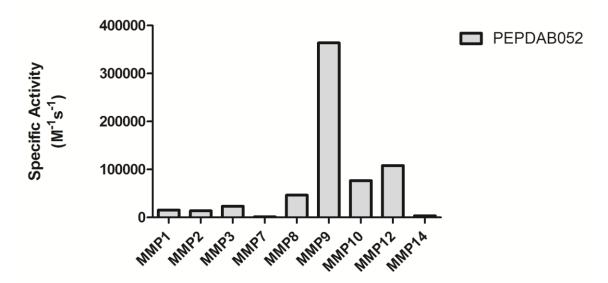
Appearance: Red solution

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



References: <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger. Integrative Biology 2010; 3(4):422-38</u>



Fluorescent Substrate: Dabcyl-APFEFSAK(FAM)-NH₂

Catalog Number: PEPDAB053

Use: This fluorescent peptide substrate is used primarily to assess activity of MMPs. It

provides good selectivity since it is not processed very well by the ADAMs tested. Its specificity constant is highly selective for MMP13. It has specificity of MMP9

over MMP2.

The peptide is already dissolved in DMSO to make a stock solution of about 5mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: ~ 1509 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

Appearance: Red solution

Shipping: The peptide solution is shipped on dry ice.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.

Stability: Samples are stable up to 6 months at -70°C.

References: <u>Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Miles A Miller, Layla Barkal, Karen Jeng, Andreas Herrlich, Marcia Moss, Linda G Griffith, Douglas A Lauffenburger. Integrative Biology 2010; 3(4):422-38</u>



Fluorescent Substrate: MCA-PRYEAYKMGK(DNP)-NH₂

Catalog Number: PEPMCA

Use: This fluorescent peptide substrate is used primarily to assess activity of ADAMs

and is selective for ADAM10. The substrate is based on the TENtide sequence discussed in Biochemical Journal, Cristina I. Caescu, Grace R. Jeschke, and

Benjamin E. Turk (2009), 424(1), 79-88.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 2mM concentration. When used for in vitro assays, the substrate is often used at about $2\mu M$ concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM 10, the buffer should consist of 25mM Tris, pH 8, 6 x 10⁻⁴ Brij

detergent, and 10mM CaCl₂.

Excitation and emission wavelengths are 325 and 393 nm respectively.

Molecular Weight: 1623.8 g/mol

Purity: Greater than 94% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 30% ACN

Appearance: Yellow lyophilized powder

Counter Ion: Trifluoroacetate

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -20 °C or lower. If dissolved in

liquid (such as DMSO), aliquot into separate tubes to minimize the number of

freeze-thaw cycles.

Stability: Samples are stable up to 6 months at -20°C.

References: Biochemical Journal, Cristina I. Caescu, Grace R. Jeschke, and Benjamin E.

Turk (2009), 424(1), 79-88.



Fluorescent Substrate: Dabcyl-GGCRPAHLRDSGK(5FAM)-NH₂

Catalog Number: PEPDAB061

Use: This fluorescent peptide substrate is based on a MMP14 biosensor and used

primarily to assess activity of MMPs 1,3 and 14.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 2mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10^{-4} Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 325 and 393 nm respectively.

Molecular Weight: 1962.2 g/mol

Purity: Greater than 90% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10%TFA

Appearance: Red lyophilized powder

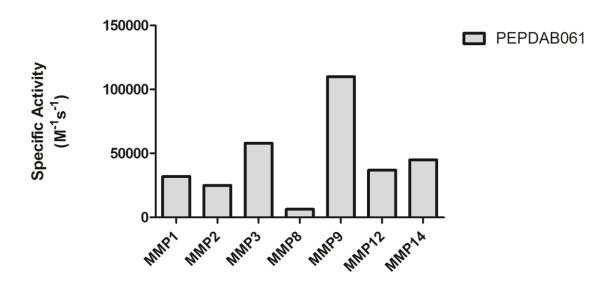
Counter Ion: Trifluoroacetate

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C or lower. If dissolved in

liquid (such as DMSO), aliquot into separate tubes to minimize the number of

freeze-thaw cycles.



References: Cancer Research, Ouyang, M. et al. (2010), 70(6), 2004-2012.



Fluorescent Substrate: Acetyl-dArg(3)-dGlu(3)-hexaminoyl-K(Dabcyl)-PRYEAYKMGK(5FAM)-C-NH₂

Catalog Number: PEPDAB063

Use: This fluorescent peptide substrate can be used to assess activity of ADAM10,

and may be specific for ADAM10. The substrate has a charged tail which is intended to keep it outside the cell so that proteolysis will only occur on the cell

surface.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAM10, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 10 mM CaCl₂, and 0.003% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 3093.5 g/mol

Purity: Greater than 94% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water with 10% Formic acid

Appearance: Red lyophilized powder

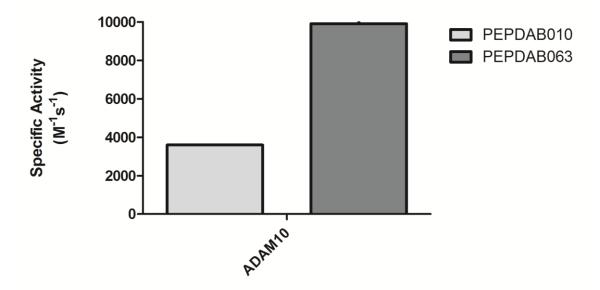
Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.





References: Biochemical Journal, Cristina I. Caescu, Grace R. Jeschke, and Benjamin E. Turk (2009), 424(1), 79-88.



Fluorescent Substrate: Dabcyl-PRAAAHomopheTSPK(5FAM)-NH₂

Catalog Number: PEPDAB064

Use: This fluorescent peptide substrate is based on the TACEtide peptide used in

Biochemical Journal, Cristina I. Caescu, Grace R. Jeschke, and Benjamin E. Turk (2009), 424(1), 79-88. and is the most sensitive substrate we have for

ADAM17.

Typically, the peptide is dissolved in DMSO to make a stock solution of about 10mM concentration. When used for in vitro assays, the substrate is often used at about $10\mu M$ concentration. Remember to keep the DMSO concentration in the

final reaction at 1% or below, to avoid DMSO effects on the reaction, and remember to have an equivalent percentage of DMSO in the background wells.

For use with ADAMs, the buffer should consist of 25mM Tris, pH 8, 6 x 10⁻⁴ Brij detergent, and 10mM CaCl₂. If used with ADAM17 or ADAM10, the CaCl₂ is not

required.

For use with the MMPs, the buffer should contain 50 mM Tris, pH 7.5, 150 mM

NaCl, 2 mM CaCl₂, 5 µM ZnSO₄, and 0.01% Brij-35

Excitation and emission wavelengths are 485 and 530 nm respectively.

Molecular Weight: 1668.5 g/mol

Purity: Greater than 95% as assessed by HPLC and Mass Spectrometry.

Solubility: 1 mg/ml in water

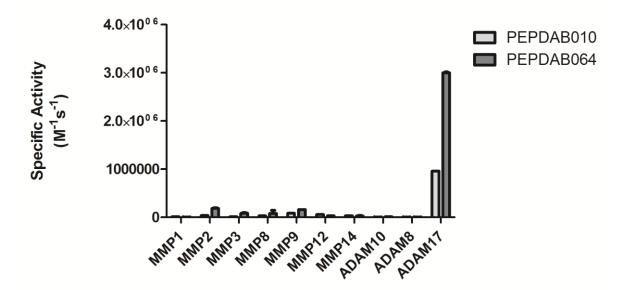
Appearance: Red lyophilized powder

Shipping: The peptide powder is shipped at room temperature.

Storage: Upon receiving, the peptide should be stored at -70 °C. Avoid repeated freeze-

thaw cycles. If dissolved in liquid (such as DMSO), aliquot into separate tubes to

minimize the number of freeze-thaw cycles.



References: Biochemical Journal, Cristina I. Caescu, Grace R. Jeschke, and Benjamin E. Turk (2009), 424(1), 79-88.

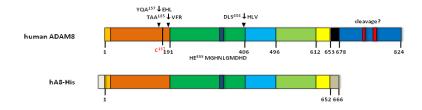


Recombinant Enzyme: Human ADAM8

Catalog Number: **ENZHADAM8**

Source: Human HEK293T cell line

Met1-Ala666 (Acc. No. AAI15405)

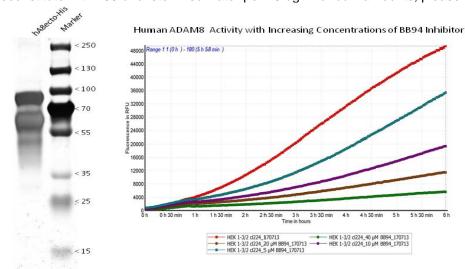


Predicted Molecular Mass:

Pro-ADAM8 ecto: ~ 80 kDa
Mature ADAM8 ecto: ~ 60 kDa

Purity: >90 %, by SDS-PAGE under reducing conditions and visualised by silver stain (see below).

Formulation: Recombinant ADAM8 is supplied as lyophilized protein in aliquots of 10 or 20 ug; reconstitute with 100 ul of sterilized water per 10 ug. For bulk amounts, please inquire.



Activity Assay Protocol:

- Assay Buffer: 50 mM Tris-HCl, 10 mM CaCl₂, 150 mM NaCl, pH 7.5 (TCN)
- 10-50 ng of recombinant ADAM8



Fluorogenic substrate - BioZyme Catalog Number PEPDAB013
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