PRODUCT DATA SHEET

MMP substrate (chromogenic)

BML-P125

Sensitive chromogenic MMP substrate

Product Number/Sizes

BML-P125-0005	5 mg

Chromogenic substrate for continuous spectrophotometric assay of most matrix metalloproteinases, and TACE (ADAM17). The MMP cleavage site peptide bond is replaced by a thioester bond in this peptide. Hydrolysis of this bond by an MMP produces a sulfhydryl group, which reacts with DTNB [5,5'-dithiobis(2-nitrobenzoic acid), Ellman's Reagent] to form 2-nitro-5-thiobenzoic acid, which can be detected by its absorbance at 412 nm.

Product Details

ALTERNATIVE NAME:	Matrix metalloproteinase substrate, Ac-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC_2H_5
SEQUENCE:	Ac-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC_2H_5
MW:	655.9
FORMULATION:	Lyophilized.
PURITY:	≥97% (HPLC)
APPLICATION NOTES:	Can be used for assay with human collagen cells.
SOLUBILITY:	Soluble in DMF (100mM) or DMSO (100mM).
SHIPPING:	Blue Ice
LONG TERM STORAGE:	-20°C
HANDLING:	After reconstitution, store at -20°C or -70°C.
PROTOCOL:	**Suggested protocol for MMP activity assay with thiopeptide substrate (Prod. No. BML-P125)**

- Dissolve thiopeptide with DMSO or DMF for a 20-50 mM stock solution. To ensure accurate concentration is achieved, both peptide purity and content need to be taken into account. Here is an example calculating the amount of DMSO needed to dissolve 5 mg BML-P125 to 50mM, when its purity is 97% and content is 93.5%:
  (mol/655.9g)x(1x10^3 mmol/mol)x(L/50mmol)x(1x10^6µl/L)x(g/1x10^3mg)x(0.935x5mg)x(0.97) =138.3µl DMSO.Store at -20°C in aliquots.
- Prepare 10X assay buffer:
  500mM HEPES
  100mM $CaCl_2$
  0.5% Brij-35
  10mM DTNB [5,5′-dithiobis(2-nitrobenzoic acid)]
  pH to 7.0
  Store at 4°C in a dark container.

  For MMP-2 add 100mM $ZnCl_2$to the 10X buffer, and substitute 500mM MOPS for HEPES. For MMP-3, substitute 500mM MES (2-[N-morpholino]ethane-sulfonic acid) for HEPES and pH to 6.0.
- Because optimal amounts of thiopeptide and MMP will vary, it is best to initially use a range of both. Suggested concentration ranges are 0-500mM thiopeptide, and 0-50nM MMP. In the end, initial velocity should be linear with respect to enzyme concentration, and the peptide must be at a saturating concentration for endpoint assays, or well below $K_m$for continuous assays. **Note: If APMA has been used to activate a pro-MMP, it must be removed (with a G-25 column), as it will react with DTNB.** Similarly, DTT, TCEP, and b-mercaptoethanol will also interfere with the assay; these can be dialyzed out.

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- Reactions (diluted assay buffer, enzyme, and substrate) are monitored in a spectrophotometer at 412nm at 25-37°C. Either cuvette or microplate (using flat-bottomed microplates) format can used. The reaction can be stopped with 50mM EDTA if desired. If MMP inhibitors are being used, incubate MMP with inhibitor for one hour prior to assay. Do not use thiol inhibitors with this substrate; they too will react with DTNB.

NOTE: This protocol serves as a guide only. Exact assay conditions must be determined by the user.

Related products also available from Enzo Lifesciences include fluorogenic MMP substrates, recombinant and purified MMPs, MMP inhibitors, and MMP inhibitor screening kits.

REGULATORY STATUS: RUO - Research Use Only

Product Literature References

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