

Pefachrome[®] Col

- Application:** Chromogenic peptide substrate for the determination of *Clostridium histolyticum* collagenase activity.
- Principle:** Collagenase cleaves off Gly-Pro-pNA from the chromogenic peptide substrate Cbo-Gly-Pro-Leu-Gly-Pro-pNA. After thermal denaturation of collagenase pNA is released from Gly-Pro-pNA by leucine aminopeptidase.
- a) Cbo-Gly-Pro-Leu-Gly-Pro-pNA + Collagenase ==> Gly-Pro-pNA
b) Gly-Pro-pNA + Leucine aminopeptidase ==> Gly-Pro + pNA
- Formula:** Cbo-Gly-Pro-Leu-Gly-Pro-pNA **MW:** 693,7
- Storage:** May be used by the expiry date given on the label when stored unopened, protected from moisture, in the dark, 2-8°C. Avoid contamination of the reagents by micro-organisms. Shipment of product does not require cooling during the time of transportation.
- Reagents:** Substrate: Pefachrome[®] Col, stock solution 25 mM in DMSO
Enzymes: Collagenase (Sigma)
Cytosolic Leucine aminopeptidase (Fluka); diluted to a concentration of 100 units/ml in buffer
Buffer: 100 mM Tris/HCl, 100 mM NaCl, 10 mM CaCl₂, 0.1% (m/V) Brij 35, pH 7.5
- Assay:** Suggested protocol for the determination of bacterial collagenase:
- Step1:
- 0.005 ml Collagenase (1000 units/ml)
0.500 ml Buffer
0.500 ml Pefachrome[®] Col 0.2 mM (stock solution diluted with buffer)
==> incubation for 5 minutes at 37° C, then the collagenase is inactivated at 80 °C for 5 minutes
- Step2:
- 0.040 ml Leucine aminopeptidase (100 U/ml); final assay concentration 4 U/ml
==> after 30 minutes of incubation the OD-value at 405 nm is recorded.
Remark: Under the given assay conditions the OD_{405nm}-value should typically increase by 0.1 units compared to the blank.
- References:** short communication
Schreier T, Imfeld D, Meier J. New chromogenic assay for the detection of *Clostridium histol.* collagenase
- Package size:** Vial containing 25 mg **Code:** 090-30
Bulk [g]

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.