3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

27672/27670 Collagenase Substrate Kit (for quantitative Collagenase-Determination)

Components:
Component A (Sigma 27673):
Collagenase-Substrate: Carbobenzoxy-Gly-Pro-Gly-Gly-Pro-Ala-OH
Molecular Weight 588.61 g/mol
CAS Number 13075-38-2

Component B (Sigma 27674):
Standard: Gly-Pro-Ala
Molecular Weight 243.26 g/mol
CAS Number 837-83-2

Enzymatic Assay of Collagenase

The component A (syntethic substrate) is hydrolysed by the collagenase to Carbobenzoxy-Gly-Pro-Gly and Gly-Pro-Ala (component B) which reacts with ninhydrin and can be determined with spectroscopic method.

Principle:

Carbobenzoxy-Gly-Pro-Gly-Gly-Pro-Ala (= Component A) —Collagenase→ Carbobenzoxy-Gly-Pro-Gly + Gly-Pro-Ala (= Component B)

Abbreviations used:
Z = Carbobenzoxy

Conditions: Incubation T = 37°C, Incubation volume 2.5 ml, pH = 6.3, Measure T = 25°C, Measure volume 5.0 ml, A_{565nm}, Light path = 1 cm
Method: Spectrophotometric Stop Rate Determination

Reagents:

		Sigma No.
1.	Citric Acid anhydrous, BioChemika Ultra	27487
2.	Calcium acetate, BioChemika Ultra	21056
3.	Sodium hydroxide, MicroSeiect	S8045
4.	Trichloric Acid, BioChemika Ultra	91228
5.	Collagenase	
6.	Ninhydrin	151173
7.	2-Methoxyethanol	33457
8.	Tin(II) chloride dihydrate, puriss p.a.	31669
9.	Sodium acetate, anhydrous, puriss.p.a.	32319
10.	Acetic acid puriss.p.a.	33209
11.	1-Propanol	402893
12.	Argon	
13.	Z-Gly-Pro-Gly-Gly-Pro-Ala Chromophore-Substrate (Component A)	27673
14.	Gly-Pro-Ala Standard (Component B)	27674

Solutions:

A) Citrate Buffer; 0,1 mol/l, pH 6,3 contains Calcium acetate 0,01 mol/l
- Prepare by dissolving 9.62 g Citric Acid and 0.79 g Calcium Acetate in ca. 300 ml H_2O distilled. Adjust the pH with NaOH >2 mol/l to 6.3. Bring to a total volume of 500 ml with H_2O distilled.

B) Calcium acetate Solution; 0,01 mol/l:
- Dissolve 158 mg Calcium acetate in 100 ml H_2O distilled

C) Collagenase-Enzyme-Solution; about 30 U/ml
- 0.68 mg Collagenase in 25 ml Calcium acetate Solution (B)

D) Trichloric Acid Solution; 1 mol/l:
- Dissolve 1.63 g in 25 ml with H_2O distilled.

E) Sodium Acetate Buffer; 4 mol/l, pH 6.3:
- Prepare by dissolving 16.4 g Sodium acetate in ca. 30 ml H_2O distilled and adjust pH to 6.3 with acetic acid. Bring to a total volume of 50 ml with H_2O distilled.

F) Ninhydrin Reagent-Solution:
- Dissolve 2.0 g Ninhydrin in 75 ml 2-Methoxyethanol, add 40 mg Tin(II) chloride dihydrate and bing to 100 ml with Sodium Acetate Buffer (E). It is a orange-yellow solution. Blanket solution with Argon and store about one day in the fridge. Colour of solution change to yellow.

G) Propanol/H_2O distilled; 50%
- Bring 25 ml Propanol to an end volume of 50 ml with H_2O distilled

H) Collagenase-Substrate (Componente A) Solution; Z-Gly-Pro-Gly-Gly-Pro-Ala-OH, 0.233 mg/ml
- Prepare by dissolving 11.5 mg of Z-Gly-Pro-Gly-Gly-Pro-Ala-OH, Sigma 27673, in 50 ml of Citrate Buffer (A).

I) Collagenase-Substrate-Standard (Componente B) Solution; Gly-Pro-Ala, 125 µg/ml
- Dissolve the different concentration in Citrate Buffer (A)
  2.500 mg in 20 ml → 30 µg/ 5 ml in Test

Stability and Storage of Solutions:
Store all solutions at 4°C. Solution A is about 1 week, solutions B, C, D, E and G for some weeks stable. Solution F should be prepared at least one day before use. Solution H and I should be prepared immediately before use.

Procedure:

Step 1:
Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank	Standard
Substrate Solution (Component A)	2.0	2.0	-
Standard Solution (Component B)	-	-	2.0
Calcium acetate	-	0.5	0.5
Collagenase Solution	0.5	-	-

Immediately mix by swirling and incubate for exactly 5 minutes at 37°C. After incubation give 0.3 ml into a 5 ml graduated flask.

Step 2:
Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank	Standard
Mixture from Step 1	0.3	0.3	0.3
Citrate Buffer	0.1	0.1	0.1

Mix immediately after addition of the buffer.

Step 3:
Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank	Standard
Mixture from Step 2	0.4	0.4	0.4
Ninhydrin Reagent-Solution	1.0	1.0	1.0

Mix well and heat 20 min. at 100°C. Cool in water bath to room temperature and bring to 5 ml total volume with Propanol/H_2O solution. Transfer the solutions to suitable cuvettes and record the A_{565nm} using a suitable spectrophotometer.

Calculations:

$$Units/ml = \frac{(A_{565nm}Test - A_{565nm}Blank)2.5 \cdot df}{5 \cdot 3.784 \cdot 0.3 \cdot 0.5}$$

2.5 [ml] = Total volume of assay in Step 1
df = Dilution factor
5 [min.] = Incubation time
3.784 [1/µmol] = extinction coefficient of 1 micromol of Gly-Pro-Ala in 5ml and 1 cm light path
(ε_{565nm} = 18.92 cm^2/µmol)
0.3 [ml] = Volume of reaction mixture in Step 1 used in Step 2+3
0.5 [ml] = Volume of enzyme used in Step 1 units/ml enzyme

$$\text{Units/mg solid} = \frac{\text{mg solid}}{\text{ml enzyme}}$$

Units Definition:
One unit will liberate 1 µmol of Gly-Pro-Ala from Z-Gly-Pro-Gly-Gly-Pro-Ala (Sigma 27673) in 1 minute at pH 6.3 at 37°C.

Note: The standard is to check the extinction coefficient (ε_{565nm} = 18.92 cm^2/µmol).

References:

1. W. Grassmann, A. Nording, Z. Physiol. Chemie 322, 267 (1960)

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.