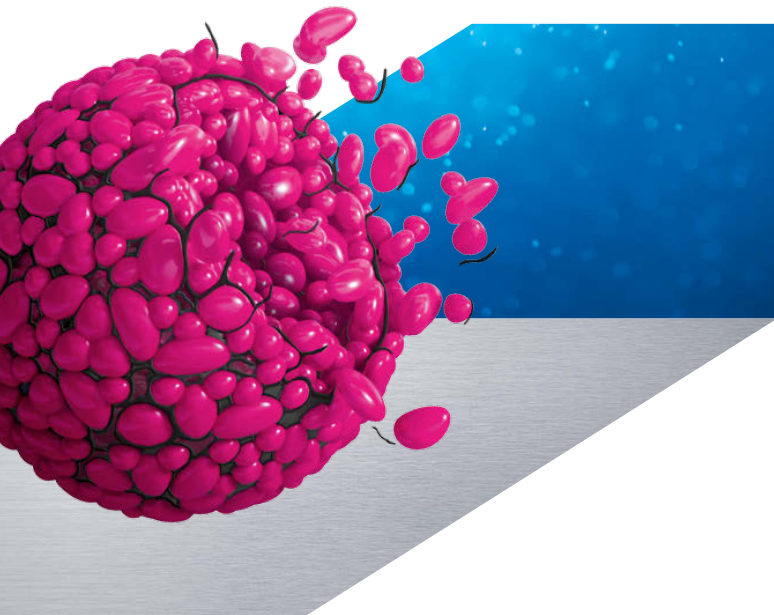


Collagenase Activity Test Kit

Fast results with high reliability

When using proteases for treatment of cells or proteins, the enzymatic activity needs to be determined in the process optimization and for process control. This is essential to develop an efficient protocol that yields the highest possible number of viable cells for further use.

The Collagenase Activity Test kit provides quantitative determination of the activity of Collagenase I, Collagenase II, and blends thereof (Liberase Enzyme Blends from Roche). The test is based on the enzymatic hydrolysis of FALGPA substrate (N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala), which can be measured photometrically by a decrease in absorbance at 340 nm. The kit is designed for testing in 96-well flat-bottom plates with a plate reader suitable for dynamic absorbance measurements.



The Collagenase Activity Test kit features:

- Reliability**
 Accurate and reproducible results due to included calibration standards and high lot-to-lot consistency of the kit.
- Convenience**
 Solutions of FALGPA substrate and Collagenase standards are ready-to-use; no manual preparation of solutions or extraction of dyes needed.
- High sensitivity**
 Limit of detection: 0.32 FALGPA Units/ml.
- Fast turnaround time**
 Time-to-result: 30 minutes.

Test characteristics

Measuring range	0.32 – 1.15 FALGPA Units/ml ¹
Precision	≤ 15% (typically < 10%)
Specificity	Collagenases I and II from <i>Clostridium histolyticum</i>
Assay time	30 min approximately
Test format	96-well plate, absorption at 340 nm

¹ Definition of FALGPA Units: 1 U catalyzes the hydrolysis of 1.0 μmole of N-(3-[2-furyl] acryloyl)-L-leucylglycyl-L-prolyl-L-alanine (FALGPA) per minute at 25°C and pH 8.8 in the presence of calcium ions (Van Wart, H.E., et al., Analytical Biochemistry, 113: 356-365, 1981).

Test procedure

In every test run, the Collagenase Standards from the kit are run on the same plate for calibration of the test. 5 µl of FALGPA Substrate are added to 95 µl of the samples and calibrators, mixed, and measured on a plate reader for 10 min. The activities of the samples are calculated by linear regression of the calibrator values.

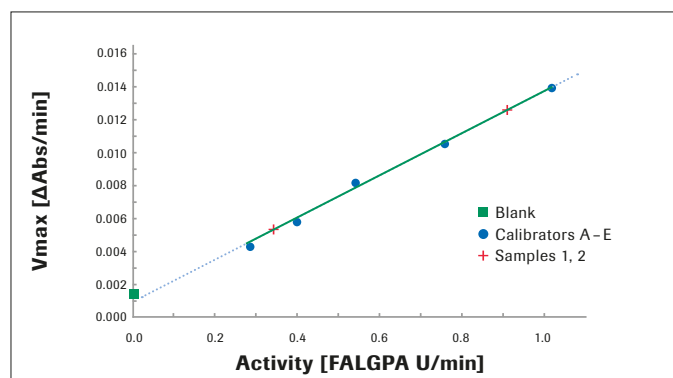


Figure: Example of a test run with linear fit of the calibrator values and two samples for determination. The bold line represents the linear test range defined by the Calibrators A to E. (Absorbance rates may be different in individual test runs.)

Specificity

This test is highly specific for Collagenases I and II from *Clostridium histolyticum*. No influence on the test result could be observed by the following proteases:

- Thermolysin
- Trypsin
- Chymotrypsin
- Dispase
- Elastase

Regulatory disclaimer

*For use in quality control/manufacturing process only.

** Blended proteolytic enzyme for tissue dissociation. For further processing only.

*** For life science research only. Not for use in diagnostic procedures.

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Comparability

The FALGPA Units of this test are proportional to other units for collagenase activity determination like Wünsch Units². The correlation between the units is different for Collagenase type I and II.

² Definition of Wünsch Units: 1 U catalyzes the hydrolysis of 1 µmole 4-phenylazobenzyl-oxycarbonyl-L-prolyl-Leucyl-glycyl-L-prolyl-D-arginine per minute at 25 °C, pH 7.1 (Wünsch, E., et al., Hoppe-Seyler's Z. Physic Chem. 333, 149 – 51, 1963).

Conversion factors from Wünsch Units to FALGPA Units:

Enzyme	Factor
Collagenase I	9.1
Collagenase II	2.7
Liberase Blend	3.0

Ordering Information

Product	Catalog number
Collagenase Activity Test *, 96 reactions	08 074 461 001
Related products	
Residual Protein Liberase Kit *, 96 reactions	07 758 693 001
Liberase MNP-S **, 35 mg	05 578 566 001
Liberase MNP-S GMP Grade **, 5 mg	06 297 790 001
Liberase MTF C/T GMP Grade **, 2 x 500 mg	05 339 880 001
Liberase T-Flex Research Grade***, 500 mg	05 989 132 001