

## Activity assay for Granzyme A (human) (recombinant)

### Basics

Molecular weight: 25816.9 D (monomer)

Extinction coefficient: 13800 mol/l<sup>-1</sup> x cm<sup>-1</sup> (Wilharm E et al. 1999 JBC 274:27331-7)

### Definition of activity

One unit hydrolyzes 1 nmol Z-Lys-SBzl per minute in 0.05 M Tris, 0.15 M NaCl, 0.01% Triton™ X-100, 0.3 mM Z-Lys-SBzl, 0.3 mM DTNB at pH 8.0 and 25° C.

### Reagents and instruments

Photometer with 25°C tempered cuvette holder, cuvette with 1cm path length and 405 nm filter.

Assay buffer: 0.05 M Tris, 0.15 M NaCl, 0.01% Triton X-100, pH 8.0 at 25° C, aliquoted and stored frozen.

Substrate: Z-Lys-SBzl•HCl (M-1300 Bachem) 45 mM in H<sub>2</sub>O, aliquoted and stored frozen.

Ellman's Reagent (Prod. No. ALX-400-034) 20 mM in DMSO

Enzyme: Granzyme A (Prod. No. ALX-201-118) 0.2 mg / ml (7747 nM monomer), aliquoted and stored frozen.

### Performance

Dilute substrate to 1.2 mM (1:37.5) in assay buffer.

Dilute DTNB to 1.2 mM (1:16.67) in assay buffer.

Dilute enzyme to 20 nM monomer (1:387.34) in assay buffer.

Mix equal volumes of substrate dilution and DTNB dilution.

Mix equal volumes of substrate/DTNB mixture (tempered to 25°C) and enzyme dilution (tempered to 25°C) in a 25°C tempered cuvette and **immediately** measure the increase of absorbance at 405 nm over the first two minutes ( $\Delta m_{Ext}/2 \text{ min}$ ).

### Calculation

$\Delta m_{Ext}/2 \text{ min} : 7.1254644 = \text{Specific activity of Granzyme A [U / } \mu\text{g]}$