

## Protocol for Granzyme B activity-assay

### **Materials**

Microtiter plate (2x plate)	Costar EIA/RIA Stripplate (8 well/flat bottom); High binding type I (art.no.2592)
antibody stock (2x 30 µl/vial)	anti-Granzyme B, 1 mg/ml frozen stock solution <i>Store at -20°C, avoid repeated freeze/thaw cycles</i>
Granzyme B standard (2x 10 µl/vial)	1000 ng/ml <i>Store at -70°C, avoid repeated freeze/thaw cycles</i> Standard line high 10 - 5 - 2.5 - 1.25 - 0.625 - 0.31 - 0.15 - 0 ng/ml Standard line low 0.31 - 0.15 - 0.08 - 0.04 - 0.02 - 0.01 - 0.05 - 0 ng/ml
Detection enzyme (2x 1.5 ml/vial)	Stocksolution in Granzyme B assay buffer 0.2 M HEPES, pH 7.5, 1 mM EDTA, 0.05% v/v Triton-X-100 <i>Store at -70°C, avoid repeated freeze/thaw cycles</i>
Chromogenic Substrate (2x 1 ml/vial)	Stock solution in distilled water <i>Store at -20°C</i>
PBS/T (not supplied)	0.01 M Phosphate buffer pH 7.5 containing 0.05% (v/v) Tween 20
Coat-buffer (not supplied)	NaAc buffer pH 5.5 Dissolve 1.5 g sodium-acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) in 80 ml distilled water. Adjust pH to 5.5 with glacial acetic acid, add distilled water to a volume of 100 ml
Granzyme B assay buffer (not supplied)	0.2 M HEPES/ NaOH pH 7.5 1 mM EDTA 0.05% v/v Triton-X-100

## Method

Coating of the microtiter plate with 2 µg/ml anti-Granzyme B coating solution :

Dilute the antibody stock-solution (1 mg/ml) to 2 µg/ml e.g. :

22 µl stock + 11 ml 0.11 M NaAc pH 5.5

Pipette 100 µl into the wells

Incubate overnight at 4 °C in a humidified chamber

Empty the wells

Wash the wells 4 times with PBS/T

Prepare the standard curves of Granzyme B in assaybuffer e.g.:

Standard line high 10 - 5 - 2.5 - 1.25 - 0.625 - 0.31 - 0 ng/ml

Standard line low 0.31 - 0.15 - 0.08 - 0.04 - 0.02 - 0.01 - 0.05 - 0 ng/ml

10 ng/ml = 5 µl 1000 ng/ml + 495 µl buffer

5 ng/ml = 250 µl 20 ng/ml + 250 µl buffer

2.5 ng/ml = 250 µl 10 ng/ml + 250 µl buffer

1.25 ng/ml = etc.

Dilute samples in Granzyme B assaybuffer

Apply 100 µl standard or (diluted) sample to the wells

Incubate 1 hour at room temperature while shaking the plate

Prepare detection reagent just before the wash step (calculate the right amount needed):

10 ml detection reagent:

8000 µl Granzyme B assaybuffer

1200 µl Detection enzyme

800 µl Chromogenic Substrate

Empty the wells

Wash the wells 4 times with PBS/T

Pipette 100 µl detection reagent to each well

Shake the plate for 20 seconds

Measure A405 at t = 0, cover the plate and incubate at 37 °C in a humidified chamber. After 2, 4 and 24 hours incubation (depending on the activity in the samples) measure A405 again. Since the reaction is not stopped, incubation can be resumed after a 2 or 4 h measurement. (This can be useful when samples have widely different activities)

Standard line high is suitable for 2 and 4 hours incubation (for samples with high activities to 10 ng/ml)

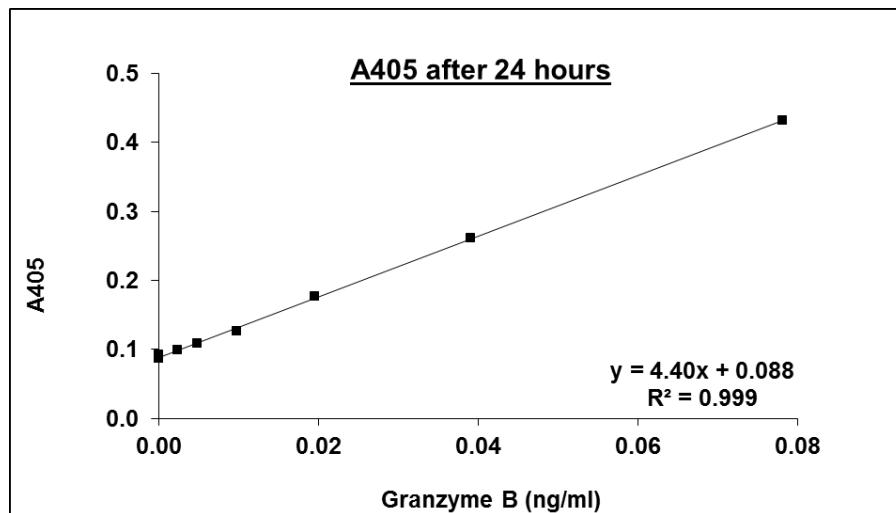
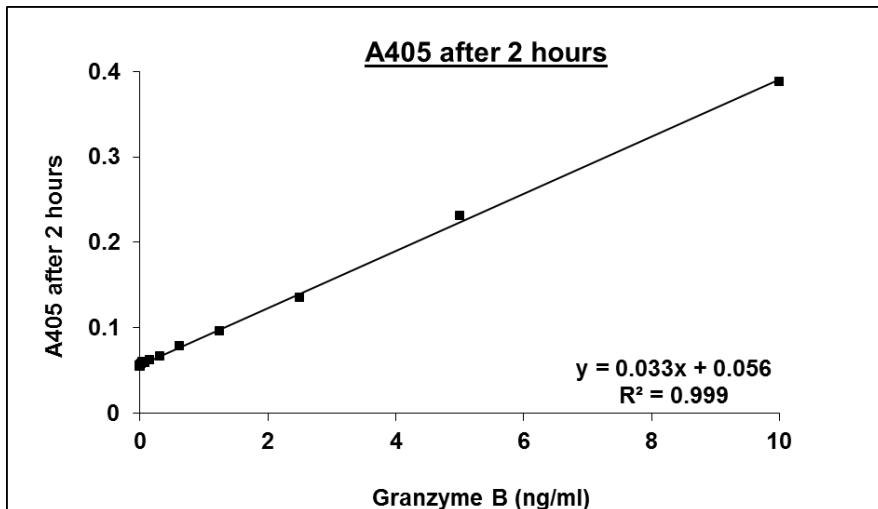
Standard line low is suitable for 24 hours incubation (for samples with low activities to 0.31 ng/ml)

Make a graph of the A405 versus the Granzyme B concentration (see Typical data)  
(do the same for 4 and 24 hours)

Draw a best-fit linearized curve through the points on the graph. Using this standard curve the A<sub>405</sub> values of the test samples can be calculated to ng/ml Granzyme B. From the sample volume used in the assay the Granzyme B concentrations can be calculated.

### Typical data

The shown data curve is provided for demonstration only. The exact A405 values can vary per experiment.



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