Protocol for Granzyme B activity-assay

Materials

Microtiter plate *(5x plate)*	Costar EIA/RIA Stripplate (8 well/flat bottom); High binding type I (art.no.2592)
antibody stock *(5x 15 µl/vial)*	anti-Granzyme B, 2 mg/ml frozen stock solution *Store at -20℃, avoid repeated freeze/thaw cycles*
Coat-buffer *(not supplied)*	NaAc buffer pH 5.5 Dissolve 1.5 g sodium-acetate ($CH_3COONa.3H_2O$) 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 assaybuffer *(not supplied)*	0.2 M HEPES/ NaOH pH 7.5 1 mM EDTA 0.05% v/v Triton-X-100
Granzyme B standard *(5x 10 µl/vial)*	1000 ng/ml *Store at -70℃, avoid repeated freeze/thaw cycles* 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 *(5x 1.5 ml/vial)*	Stocksolution in Granzyme B assaybuffer 0.2 M HEPES, pH 7.5, 1 mM EDTA, 0.05% v/v Triton-X-100 *Store at -70℃, avoid repeated freeze/thaw cycles*
Chromogenic Substrate *(5x 1 ml/vial)*	Stock solution in distilled water *Store at -20℃*
PBS/T *(not supplied)*	0.01 M Phosphate buffer pH 7.5 containing 0.05% (v/v) Tween 20

Method

Coating of the microtiter plate with 2 µg/ml anti-Granzyme B coating solution :
Dilute the antibody stock-solution (2 mg/ml) to 2 µg/ml e.g. :
11 µl stock + 11 ml 0.11 M NaAc pH 5.5
Pipette 100 µl into the wells
Incubate overnight at 4 ℃ 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

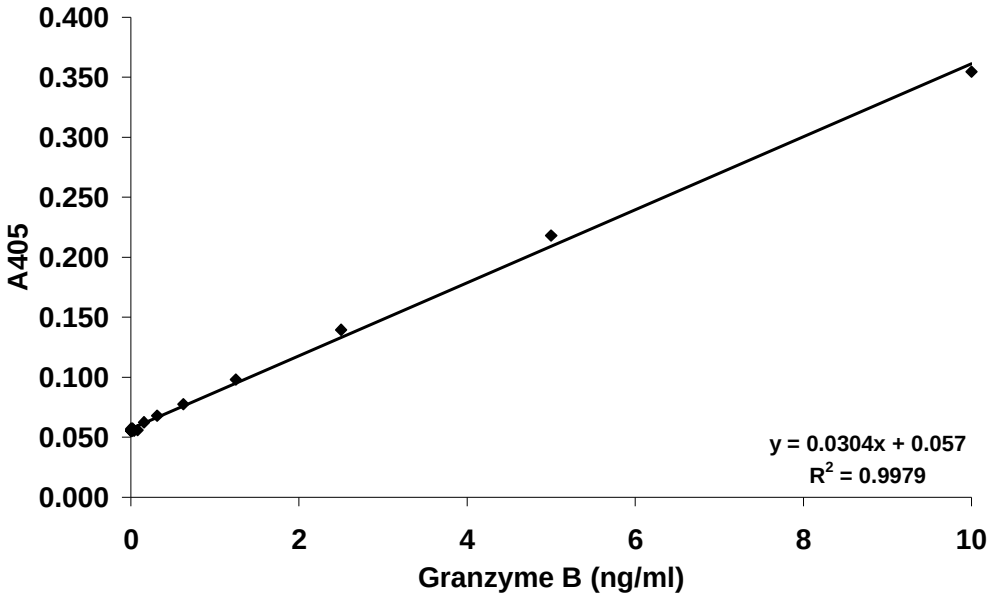
Measure A405 at t = 0, cover the plate and incubate at 37 ºC in a humidified chamber. After 2, 4 and 24 hours incubation (depending on the activity in the samples) measure A405 again. Since the reaction is not stopped, incubation can be resumed after a 2 or 4 h measurement. (This can be useful when samples 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_{405} 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

A405 after 4 hours



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