

Protocol for Granzyme B activity-assay

Materials

Microtiterplate <u>(5x plate)</u>	Costar EIA/RIA Stripplate (8 well/flat bottom); High binding typel (art.no.2592)
antibody stock <u>(5x 15 µl/vial)</u>	anti-Granzyme B, 2 mg/ml frozen stock solution <i>Store at -20°C, avoid repeated freeze/thaw cycles</i>
antibody coat-solution <u>(not supplied)</u>	11 µl antibody stock-solution (2 mg/ml) + 11 ml coat-buffer (0.11 M NaAc pH 5.5)
Coat-buffer <u>(not supplied)</u>	0.11 M NaAc buffer pH 5.5 Dissolve 15 g sodium-acetate (CH ₃ COONa.3H ₂ O) in 800 ml distilled water. Adjust pH to 5.5 with glacial acetic acid, add distilled water to a volume of 1 liter.
Granzyme B assaybuffer <u>(not supplied)</u>	0.2 M HEPES NaOH pH 7.5 1 mM EDTA 0.05% v/v Triton-X-100
Granzyme B standard <u>(5x 10 µl/vial)</u>	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 Std.line low 0.31 - 0.15 - 0.08 - 0.04 – 0.02 – 0.01 – 0.05 - 0 ng/ml
Detection enzyme <u>(5x 1.5 ml/vial)</u>	Stocksolution in 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 <u>(5x 1 ml/vial)</u>	Stocksolution in distilled water <i>Store at -20°C</i>
PBS/T <u>(not supplied)</u>	0.01 M Phosphate buffer pH 7.5 containing 0.05% (v/v)Tween 20

Method

Coating of the microtiterplate 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 °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

Std.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 and after 2, 4 and 24 hours depending on the activity in the samples.

Standard line high: 2 and 4 hours incubation (for samples with high activities)

Standard line low: 24 hours incubation (for samples with low activities)

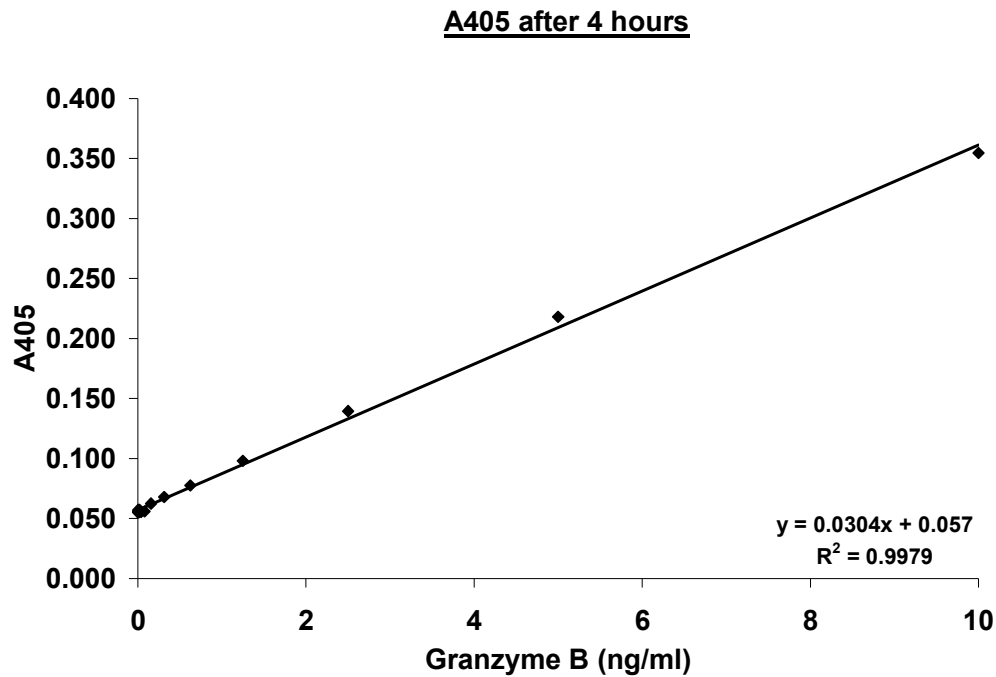
Plot the A₄₀₅ versus the Granzyme B concentration (see Typical data)

(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 A₄₀₅ 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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