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Detecting degranulation via hexosaminidase assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the quantification of hexosaminidase in cell culture supernatants via an enzyme activity assay. You can use hexosaminidase levels to determine whether mast cells or basophils have undergone degranulation. This protocol uses the Cell BioLabs beta-hexosaminidase assay kit (Fisher Scientific (NC1634727)).

Materials

 Beta-Hexosaminidase Assay Kit **Fisher Scientific Catalog #NC1634727**

Black 96-well microtiter plate

Plate reader

1.5 mL microcentrifuge tubes

15 mL centrifuge tubes

Cell culture supernatants (analyte)

Protocol materials

 Beta-Hexosaminidase Assay Kit **Fisher Scientific Catalog #NC1634727**

Reagent preparation

- 1 This protocol uses the  Beta-Hexosaminidase Assay Kit **Fisher Scientific Catalog #NC1634727** . If you've already prepared kit reagents, skip to "Standard and substrate preparation."
- 2 Dilute 5× assay buffer to 1× with ultrapure water. Vortex to homogeneity. Store at  Room temperature .
- 3 Dilute 10× neutralization buffer to 1× with ultrapure water. Vortex to homogeneity. Store at  Room temperature .

Standard and substrate preparation

- 4 Thaw hexosaminidase positive control and 10× substrate  On ice .
- 5 Just prior to use, prepare  35 µL positive control at  5 µg/mL (10-fold dilution) in 1× assay buffer.
- 6 Prepare control solutions as follows:

	A	B	C	D
	Control	Volume 5 µg/mL hexosaminidase (µL)	Volume buffer (µL)	Volume per well (µL)
	500 ng/mL hexosaminidase	17.5	157.5	50
	250 ng/mL hexosaminidase	8.75	166.25	50
	125 ng/mL hexosaminidase	4.38	170.62	50
	0 ng/mL hexosaminidase	0	175	50
	Blank	0	350	100



- 7 Dilute 10× substrate to 1× in assay buffer. Vortex to mix. Prepare only enough 1× substrate for immediate use ( 50 µL per well, not including blanks).

Sample preparation and analysis

15m

- 8 Perform assay in technical triplicate.
- 9 Add  50 µL of each sample or control per well to appropriate wells of a black, 96-well microtiter plate (except blank wells, which get  100 µL).
- 10 Add  50 µL 1× substrate per well to all wells except the blank.
- 11 Incubate at  37 °C for  00:15:00 , protected from light.
- 12 Add  100 µL of 1× neutralization buffer to each well.
- 13 Read each well's fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

Note

DMSO absorbs significantly at 365 nm. If using DMSO, ensure that its concentration is normalized across all samples.

Protocol references

Manufacturer protocol:

https://www.cellbiolabs.com/sites/default/files/MET-5095-beta-hexosaminidase-activity-assay-kit_0.pdf