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ProductInformation

Phospholipase A2 Group GXII, (PLA_2 –GXII)
Mouse, Recombinant, Expressed in *E.coli*

Product Number **P3244**
Storage Temperature -20°C

Product Description

The PLA_2–GXII is a novel murine 20kDa protein, which contains at least two alternative spliced forms. PLA_2-GXII is expressed in *E. coli* as a soluble MBP fusion protein and purified under non-denaturing conditions. PLA_2-GXII runs on SDS-PAGE with an apparent molecular weight of approximately 65 kDa. The catalytic domain of PLA_2–GXII is cysteine-rich. The enzymatic activity of the protein is optimal at pH 8.0 and in low millimolar calcium concentration. PLA_2–GXII is preferentially expressed in type 2 helper (Th2) cells and might participate in T cell immune response through release of immediate secondary signals and generation of downstream eicosanoids. Its role in the cell signaling cascade is still not known [(1)]. The PLA_2-GXII binds to Maltoheptose (Sigma Product. Number M 9676).

Reagents

This product is sold as a lyophilized powder essentially salt free.

Precautions and Disclaimer

This product is for laboratory research only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the vial with deionized water. The PLA_2-GXII is soluble in water at 0.5 mg/mL. The product can be also reconstituted in 25 mM Tris, pH 8.0.

Storage/Stability

Store at –20°C. Upon initial thawing, for extended storage, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing of the peptide.

Procedure

Principle of assay:
The assay is a liposome based assay[1-4]. Liposomes are prepared from the radioactive phospholipid (Phosphatidylethanolamine (PE), L-α-1-palmitoyl-2-arachidonyl, [arachidonyl-1-^{14}C] (^{14}C-PE)), which is the PLA_2-GXII substrate. Following ^{14}C–2-arachidonic acid hydrolyses by the PLA_2-GXII, the lipid is extracted by n-heptane. The extracted radioactivity is counted in liquid scintillation counter.

Materials and reagents:

- Phosphatidylethanolamine (PE), L-α-1-palmitoyl-2-arachidonyl, [arachidonyl-1-^{14}C]
- Calcium chloride (Sigma Product Number C 1016)
- TRIS base (Sigma Product Number T 1503)
- 2-propanol
- Hydrochloric acid, 36%
- n-heptane
- Albumin (Sigma Product Number A 8022)
- Silica gel 60, 0.015-0.040 mm
- Argon or nitrogen gas
- Bath sonicator
- Rotator wheel
- Glass tubes 12X75 mm
- Eppendorf 2 mL tubes with safe lock
- Eppendorf 1.5 mL tubes with safe lock
- Scintillation liquid
- Scintillation tubes
- Caps for scintillation tubes
- Liquid scintillation counter

Reaction Buffer X1.25:
1.25 mM $CaCl_2$, 10 mM Tris-HCl, pH 8.0
1 mL is sufficient for 10 reactions.

Radioactive material (^{14}C-PE):
From phosphatidylethanolamine (PE), L-α-1-palmitoyl-2-arachidonyl, [arachidonyl-1-^{14}C] as stock solution of 1 mM*, 4.5 µL should be added to 1 mL of 1.25X reaction buffer (4.5 µM). The final concentration of ^{14}C-PE in the assay is 3.6 µM.

Calculation of radioactive material concentration (example):
The radioactive material is sold at 50 mCi/mL; in the example the specific radioactivity is 48 mCi/mmol~ 50 µCi/1 µmoL, which equals 1 µmol/1mL= 1 mM.

Stop Solution: 2-propanol: 1 M HCl 700:60
Mix 7 mL of 2-propanol with 0.6 mL of 1 M HCl. Prepare fresh for every assay.

Liposome preparation:

- Transfer the ^{14}C-PE (the radioactive material) to glass tube, and dry under nitrogen.
- Resuspend the dry material in 1 mL of 1.25X reaction buffer.
- Cover with parafilm.
- Sonicate at a bath sonicator 4X10 seconds till cloudiness appears (liposome performance).

Note: the liposomes should be prepared just before the experiment.

Sample preparation:

- Reconstitute a vial of PLA2-GXII with water.
- Determine the protein concentration.

The amounts of PLA2-GXII used in the assay are 500 ng to 1500 ng.

The sample is added in the amount 25 µl/assay.

Note: The units are calculated per 500 ng/assay results.

Dilute the PLA2-GXII to 500 ng/25 µL in water e.g. to 20 µg/mL.

BSA control: Dilute 1 mg/mL stock solution to 20 µg/mL in water.

Reaction Scheme:

	Liposomes	PLA_2-GXII	BSA
Blank	100 µL	----	25 µL
Sample1	100 µL	25 µL	----
Sample 2	100 µL	25 µL	----

Procedure:

1. Transfer 100 µL (for every reaction) of the liposomes into 2 mL Eppendorf tube according to the reaction scheme.
2. Add 25 µL of 20 µg/mL BSA to the control tube (in duplicates).
3. Add 25 µLof 20 µg/mL PLA2-GXII to the sample tube (in triplicates).
4. Close the tubes and vortex.
5. Incubate the tubes for 30 minutes at 37 ºC.
6. Spin down the reaction tube.
7. Stop the reaction by adding 625 µLof the stop solution.
8. Vortex the sample and leave it at room temperature for at least 10 minutes.
9. Add 400 µl of H_20, and 700 µl of n-heptane.
10. Vortex for 10 seconds and centrifuge at maximum speed for 10 minutes, to separate the phases.
11. Prepare a set of 1.5 mL Eppendorf tubes containing 0.35 mg (0.00035 g) silica gel. The silica gel treatment reduces the background by binding the non hydrolyzed ^{14}C-PE.
12. Transfer 500 µL from the upper phase to 1.5 mL Eppendorf tubes containing silica gel; be careful not to touch the lower phase.
13. Add 200 µL of n-heptane.
14. Vortex and rotate on a wheel for at least 10-15 minutes.
15. Centrifuge the samples at 8000 g for 10 minutes.
16. Transfer the supernatant (approximately 650 µL) into scintillation tubes.
17. Add 4 mL of scintillation liquid.
18. Cap the tubes and shake well.
19. Count in liquid scintillation counter.
20. To establish the total cpm of the liposomes for one reaction, take 10 mL of liposome, add 4 mL of scintillation liquid, and count in liquid scintillation counter.

Results

Specific activity calculations:

- **nmol PE/assay**- every reaction tube contains 3.6 µM of radioactive material, or 3.6 µmol/mL X 0.125mL (volume per tube) =0.45 nmol/test.
- **Total cpm**-cpm of the 10 mL of liposomes X 10.
- **Specific radioactivity of the reaction (SR)** cpm/nmol -Total cpm/ nmol
- **Time** (t)-reaction time in minutes.

Product Profile

	cpm	cpm Sample minus - Control
Control	1026	
Sample 1	3913	2887
Sample 2	3617	2591
Sample 3	3893	2867
Average cpm (samples 1-3)		**2782**

10 µL of liposomes -3500cpm
Time-30 minutes
Protein-500 ng=0.0005 mg

Total cpm- 3500 X 10=35,000

S.R.= $\frac{35000}{0.45}$ = 77,777 cpm/nmol

Specific activity= $\frac{2782}{77777} \times \frac{1}{30} \times \frac{1}{0.0005} = 2.30$ nmol/min/mgP

Note: The PLA_2-GXII weight is calculated as 1/3 of the recombinant protein molecular weight.

References

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4. Vermehren, C., et al., Int. J. Pharm., **214**, 93-98, (2001).

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