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Version 1

Calf-Intestinal Alkaline Phosphatase Treatment in situ-Killinger 2024 V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Alpha-synuclein phosphorylated at serine 129 (PSER129) occurs in two pools, non-aggregated (physiological) and aggregated (disease). This protocol allows for the selective dephosphorylation of non-aggregated PSER129 and enhances the specificity and sensitivity immunodetection of aggregated PSER129. Thus, this protocol can be used to differentiate physiological from aggregated PSER129.

Materials

Dilution media:

	A	B
	Nacl	150 mM
	Tris-HCl, pH 7.4	50 mM
	Triton-X100	0.5%

CIAP buffer:

	A	B
	Nacl	100 mM
	Tris- Hcl	50 mM
	Mgcl2, pH 7.9	10 mM

-  Alkaline Phosphatase Calf Intestinal (HC), 1,000u **Promega Catalog #M2825**

Day 1

1d 1h 10m

1 Wash free-floating tissue (3 × 10 minutes) in dilution media.



▪ Dilution media:

	A	B
	NaCl	150 mM
	Tris-HCl pH 7.4	50 mM
	Triton-X100	0.5%

1.1 Wash free-floating tissue  00:10:00 in dilution media (1/3).

10m



1.2 Wash free-floating tissue  00:10:00 in dilution media (2/3).

10m



1.3 Wash free-floating tissue  00:10:00 in dilution media (3/3).

10m



2 Incubate the samples with 1% Triton X-100 in DM for  00:10:00 .

10m



3 Wash in DM  00:10:00 .

10m



4 Wash the tissues in CIAP buffer (2×10 minutes).



▪ CIAP buffer:

	A	B
	NaCl	100 mM
	Tris- HCl	50 mM



A	B
MgCl ₂ , pH 7.9	10 mM

Autoclave and store Room temperature .

4.1 Wash the tissues in CIAP buffer 00:10:00 (1/2).

10m



4.2 Wash the tissues in CIAP buffer 00:10:00 (2/2).

10m



5 Incubate the tissues with CIAP at a dilution of 1:333 for 24:00:00 at 37 °C on a shaker.

1d



- CIAP concentration per bottle: 20 u/μl (Promega, Cat.# M2825).

- In 500 μL CIAP buffer, add 1.5 μL CIAP (30 units).

Day 2

3h 20m

6 Wash in DM (2 × 10 minutes).



6.1 Wash in DM 00:10:00 (1/2).

10m



6.2 Wash in DM 00:10:00 (2/2).

10m



7 Heat water bath to 80 °C - 85 °C 01:30:00 before the antigen retrieval step.

1h 30m

8 Place the dish containing sodium citrate buffer in the water bath and heat it for 00:10:00 .

10m



- Sodium Citrate Buffer, pH 6.0 (1L): 2.94 g Sodium citrate-Trisodium salt (Dihydrate) in 1000 mL DI water. pH 6.0. Add 0.5 mL Tween-20. Mix well.

9 Wash the tissues in sodium citrate buffer 00:10:00 .

10m



10 Incubate the tissues in the heated sodium citrate buffer for 00:30:00 .

30m



11 Cool the dish containing tissues to Room temperature (at least 00:20:00).

20m

12 Wash in DM for 10 min x 2 times.



12.1 Wash in DM for 00:10:00 (1/2).

10m



12.2 Wash in DM for 00:10:00 (2/2) .

10m



13 Tissues are now ready for downstream assays.