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Calf-Intestinal Alkaline Phosphatase Treatment in situ-Killinger 2024 V.2

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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.

For subsequent detection of non-aggregated-(physiological PS129) we have observed that the blocking buffer component bovine serum album (BSA) fraction V from several sources interferes with physiological PS129 detection. We have had success with MP Biomedicals AlbumiNZ Protease-reduced BSA, as well as omitting BSA from blocking buffers. We have not determined the source of interference, but it may include low level proteases or phosphatases.

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
	Mgcl2, 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.