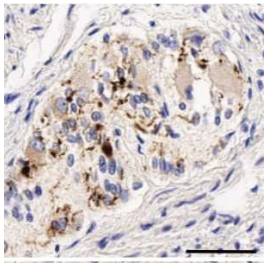
Aug 28, 2024 Version 1

Automated immunodetection of proteinase-K-resistant Lewy Pathology in FFPE human colon tissue V.1



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Protocol status: Working
We use this protocol and it's working

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Abstract

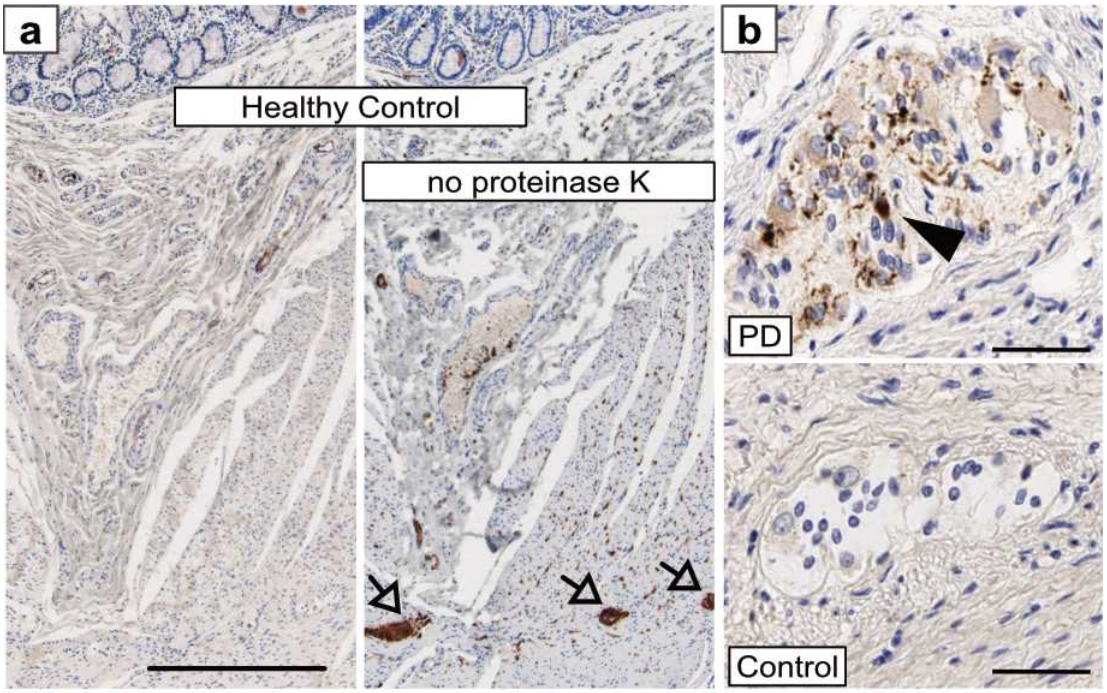
Automated detection of gut Lewy Pathology using Leica Bond RX processor based on Protocol F using Bond Polymer Refine Detection kit (Leica Biosystems, Cat#: DS9800). Adapted from "Method 5" as described in Beach TG et al., J Parkinsons Dis. 2016.

CITATION

Beach TG, Corbillé AG, Letournel F, Kordower JH, Kremer T, Munoz DG, Intorcia A, Hentz J, Adler CH, Sue LI, Walker J, Serrano G, Derkinderen P (2016). Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects..

LINK

https://doi.org/



a. Adjacent sections from a healthy control stained for phosphorylated α-Syn (MJFR-13, abcam ab168381) are shown with (left) and without (right) proteinase K treatment demonstrating robust detection of insoluble LP. b. Higher power view of Lewy Pathology (solid arrow) in a PD myenteric plexus ganglion. Samples obtained from Banner Health Brain and Body Donation Program. Scale bars are 50μm.

Acknowledgments

We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona, for the provision of human colon tissue and data. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinsons Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimers Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimers Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinsons Disease Consortium) and the Michael J. Fox Foundation for Parkinsons Research.

We wish to acknowledge Yunfeng Li and the Translational Pathology Core Laboratory (TPCL) at UCLA for assistance with tissue processing, IHC staining, and scanning.

Materials

Note

BOND Polymer Refine Detection Kit **Leica Biosystems Catalog #**DS9800 contains

- Peroxide Block (30 mL) 3–4% (v/v) Hydrogen peroxide.
- Polymer (30 mL) Anti-rabbit Poly-HRP-IgG (<25 µg/mL) containing 10% (v/v) animal serum in tris-buffered saline/0.1% ProClin™ 950.
- DAB Part 1 (2.4 mL) 66 mM 3,3'-Diaminobenzidine tetrahydrochloride hydrate, in a stabilizer solution.
- DAB Part B (30 mL) ≤0.1% (v/v) Hydrogen Peroxide in a stabilizer solution.
- Hematoxylin (30 mL) <0.1% Hematoxylin

Equipment

new equipment	NAME
Leica BOND RX	BRAND
3342171	SKU
https://www.leicabiosystems.com/ihc-ish-fish/ihc-ish-instruments/products/leica-bond-rx/	SPECIFICATIONS

Protocol materials

- BondTM Wash Solution 10X Concentrate **Leica Biosystems Catalog #**AR9590 In 2 steps
- Anti-Alpha-synuclein (phospho S129) antibody [MJF-R13 (8-8)] **Abcam Catalog #**AB168381 Step 5
- Histo-Clear **National Diagnostics Catalog #**HS2001GLL Step 17
- Chemical Permount Mounting Medium **Fisher Scientific Catalog #**SP15-100 Step 17
- Epitope Retrieval Solution 1 BOND **Leica Biosystems Catalog #**AR9961 Step 2
- EnVision System-HRP, Labelled Polymer (Rabbit) **Agilent Technologies Catalog #**K4003 Step 7
- BOND Polymer Refine Detection Kit **Leica Biosystems Catalog #**DS9800 Materials
- Dewax Solution **Leica Biosystems Catalog #**AR9222 Step 1
- BOND Enzyme Pretreatment Kit **Leica Biosystems Catalog #**AR9551 Step 2

1 Leica Bond RX routine factory based “Bake and Dewax” protocol.

Dewax Solution **Leica Biosystems Catalog #**AR9222

2 **Enzyme Antigen Retrieval**: Incubate in Enzyme 1 solution for 00:20:00 at 20m

Room temperature

BOND Enzyme Pretreatment Kit **Leica Biosystems Catalog #**AR9551

Note

For comparison, this step can be replaced with Heat Induced Antigen Retrieval using ER1 buffer for 00:20:00

Epitope Retrieval Solution 1 BOND **Leica Biosystems Catalog #**AR9961

3 Incubate in Peroxide Block for 00:05:00 5m

4 Incubate in Bond Wash buffer three times 00:00:00 each.

BondTM Wash Solution 10X Concentrate **Leica Biosystems Catalog #**AR9590

5 **Primary antibody:** Incubate in 1:10,000 1h

Anti-Alpha-synuclein (phospho S129) antibody [MJF-R13 (8-8)] **Abcam Catalog #**AB168381

for 01:00:00 at Room temperature

6 Incubate in Bond Wash buffer three times 00:00:00 each.

BondTM Wash Solution 10X Concentrate **Leica Biosystems Catalog #**AR9590

7 Incubate with labeled polymer 00:10:00 10m

EnVision+ System-HRP, Labelled Polymer (Rabbit) **Agilent Technologies Catalog #**K4003

8 Incubate in Bond Wash buffer three times 00:02:00 each. 2m

9 Incubate with polymer for 00:08:00 . 8m

10 Incubate in Bond Wash buffer five times 00:00:00 each.

11 Deionized water wash 00:00:00 .

12 Incubate with Mixed DAB Refine for 00:00:00 followed by Mixed DAB Refine for 00:10:00 . 10m

13 Deionized water wash for three times 00:00:00 each.

14 Incubate with Hematoxylin for 00:10:00 . 10m

15 Incubate in Bond Wash buffer three times 00:00:00 each.

16 Deionized water wash 00:00:00 .

17 Slides are dehydrated in series of alcohols, cleared with Histo-Clear **National Diagnostics Catalog #**HS2001GLL and mounted with Chemical Permount Mounting Medium **Fisher Scientific Catalog #**SP15-100

Protocol references

Beach TG, Corbillé AG, Letournel F, Kordower JH, Kremer T, Munoz DG, Intorcia A, Hentz J, Adler CH, Sue LI, Walker J, Serrano G, Derkinderen P. Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects. J Parkinsons Dis. 2016 Oct 19;6(4):761-770. doi: 10.3233/JPD-160888. PMID: 27589538; PMCID: PMC5501392.

Citations

Beach TG, Corbillé AG, Letournel F, Kordower JH, Kremer T, Munoz DG, Intorcia A, Hentz J, Adler CH, Sue LI, Walker J, Serrano G, Derkinderen P. Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects.
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