

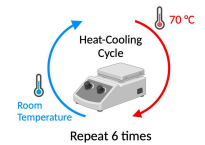
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Cyclic Heat Induced Epitope Retrieval (CHIER)

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Cyclic Heat Induced
Epitope Retrieval (CHIER)



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Protocol status: Working

We use this protocol and it's working

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Abstract

Immunolabeling is a cornerstone technique in molecular biology and pathology, providing critical insights into protein localisation and function within biological tissues. However, one of the persistent challenges in immunolabeling is the effective unmasking of epitopes, which are often concealed in formalin-fixed, paraffin-embedded (FFPE) tissue samples. While effective to an extent, traditional antigen retrieval methods have limitations in consistency, epitope preservation, and overall labelling efficiency. We introduce a novel antigen retrieval protocol, Cyclic Heat Induced Epitope Retrieval (CHIER) to address these issues.

The development of CHIER is particularly crucial for studies involving proteins that are notoriously difficult to label due to dense cross-linking or subtle epitope masking. By improving the efficiency of antigen retrieval, CHIER enhances the sensitivity and specificity of antibody binding in immunohistochemical (IHC) and immunofluorescence (IF) assays. This results in more robust staining and can facilitate the detection of densely aggregated proteins, which is essential for accurate protein localisation and quantification studies.

Significantly, CHIER demonstrated a remarkable ability to increase the detection of the protein of interest (SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin subfamily c member 2, SMARCC2), with results showing a 3x increase in detection compared to traditional methods. This enhancement in detection efficiency makes CHIER a valuable tool for researchers in diverse fields, including neurobiology, cancer research, and tissue pathology, where precise antigen detection is paramount. Introducing CHIER into these fields is expected to significantly advance our understanding of complex protein dynamics and interactions in various biological and disease contexts.

Guidelines

Heat block reaches 70° Celcius and can cause burns when skin comes into contact with the hot surface.



Materials

Tris-EDTA antigen retrieval buffer in dH₂O

- 10mM Tris Base
- 1 mM EDTA Solution

Adjust to pH 9.0

- 0.05% Tween 20

Safety warnings

- ! Heat block reaches 70° Celcius and can cause burns when skin comes into contact with the hot surface. Make sure chemical waste is disposed of according to on-site regulations.

Ethics statement

The human post-mortem brain tissue used in this project was sourced from the Neurological Foundation Human Brain Bank (Centre for Brain Research, University of Auckland, New Zealand). All brain tissue was donated upon written, informed consent from the individual(s) and/or next of kin before brain removal.

Please ensure consent is obtained, and all protocols required used are approved by the relevant Ethics Committee.



Paraffin-embedded formalin-fixed brain tissue

- 1 Human paraffin-embedded formalin-fixed brain tissue was utilised for this project. All brain tissues were washed using an ethanol (EtOH) series at Room temperature :

1h 55m

- 00:20:00 70% EtOH
- 00:20:00 80% EtOH
- 00:20:00 2 x 95% EtOH
- 00:30:00 2 x 100 % xylene

Once washed and dehydrated, the tissue was embedded in molten paraffin wax for 3 x

- 00:25:00 cycles.

Cutting parafin sections

- 2 Paraffin blocks were cut into 7 µm thick sections using a rotatory microtome (Leica Biosystem Histcore BIOCUT R) and placed into a 37 °C water bath (Leica Biosystems, Histcore & H1210). Sections were individually mounted onto charged Uber frost Printer PLUS 3000 microscope slides (Intrumentec) and placed on a slide dryer (Leica Biosystems Histcore) for 5 minutes, followed by air-drying at room temperature for a minimum of 72:00:00 .

3d

Cyclic Heat Induced Epitope Retrieval (CHIER) Hotplate

1h 10m

- 3 Mounted slides with Formalin-fixed paraffin-embedded tissue sections were placed tissue side up

- 3.1 Mounted slides with Formalin-fixed paraffin-embedded tissue sections were placed, on a 70 °C hotplate (Leica HI1220) for 00:05:00 followed by 00:05:00 at room temperature.

1h

This heat-cooling cycle was repeated six times.



Note

Incubation time and temperature are critical.



- 3.2 Immediately following the heat-cooling cycle, slides were placed on a 60 °C hotplate (Leica HI1220) for 00:30:00 .

30m

Note

If using the same hotplate then make sure the temperature drops to 60 °C before placing slides.

Dewaxing

1h 20m

4

1h 5m

Immediately following hotplate incubation, sections were submerged in

00:30:00 2 x 100% xylene baths to remove paraffin wax.

Subsequently, tissue sections were rehydrated by submerging slides in a series of alcohol baths

00:15:00 2 x 100% EtOH

00:05:00 95% EtOH

00:05:00 80% EtOH

00:05:00 75% EtOH

00:05:00 3 x distilled H₂O (dH₂O) to wash of any remaining EtOH.

Antigen retrieval in pressure cooker

1h 40m

- 5 The tissue on the slides is now ready for subsequent steps. Continue with antigen retrieval as normal, followed by blocking and subsequent antibody incubation.