Jan 18, 2024

JMN-MSMP NIA Automated Histological Staining

DOI
dx.doi.org/10.17504/protocols.io.eq2lyjb6rlx9/v1

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[1]SenNet JMN-MSMP

Cellular Senescence Net...

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DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyjb6rlx9/v1

Protocol Citation: ccherry 2024. JMN-MSMP NIA Automated Histological Staining. **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2lyjb6rlx9/v1**

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

Created: January 15, 2024

Last Modified: January 18, 2024

Protocol Integer ID: 93523

Keywords: sennet aged mouse histological staining, histological staining, staining sennet, msmp nia automated histological, aged mouse, staining, mouse, jmn

Abstract

SenNet aged mouse histological staining from NIA

Materials

Antibodies:
Recombinant Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) (recommended dilution 1:1000)
Recombinant Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542) (recommended dilution 1:400)
Recombinant Anti-p21 antibody [EPR18021] (ab188224) (recommended dilution 1:1000 with mild antigen retrieval)

Troubleshooting

Fixation

1 Take the whole carcass into a jar and fix in formalin for 24 hr

2 Wash the carcass with 70% EtOH and store in EtOH jar until histogrossing

Tissue collection:

3 Take out the ovaries together with uterus and vagina from the female samples and put into a histology cassette.

4 For the cervical spine, cut the next few millimeters down while keeping the mouse ventral down after cutting head and put into a compartment of the cassette.

5 Place the mouse on its back and use scalpel to cut in the middle of the ribcage then cut again a few vertebrae down to obtain the thoracic spine and put into the second compartment of the same cassette.

6 Cut a few millimeters below the ribcage and then cut again a few vertebrae down to obtain the lumbar spine and put into the third compartment of the same cassette.

7 Trim off any fur, skin, or extra tissue around each of the spine specimens so there is just the bone surrounded by a few millimeters of tissue.

8 For skin, take a dorsal cervical specimen and a ventral inguinal specimen

9 Cut specimens in a strip parallel to hair growth and place skin side down in a cassette. Place the closed cassettes of reproductive organs and skin samples in a small jar, and the spine sample into another jar.

Decalcification:

10 Place the spine cassette into a jar and fill with decalcification solution.

11 After 24 hr, pour out the decalcification solution into the chemical waste container and refill with fresh decalcification solution

12 After 24 hr, pour out the decalcification solution into the chemical waste container

13 Bring the jar to the sink, cover with an inverted strainer, and allow running water to run over the cassettes and rinse them of any remaining decalcification solution or formalin for 30 min.

14 After rinsing, empty out all the water and fill the jar with 70% EtOH.

15 Send the cassettes of skin, reproductive tissues and spine in jars to Oncology Tissue Services Core of Johns Hopkins University School of Medicine. The tissues are embedded in paraffin, sectioned and immunostained in the core.

Immunohistochemistry:

16 This process is performed in Oncology Tissue Services Core of Johns Hopkins University School of Medicine. Immunolabeling for p21, p16 and γH2AX is performed on formalin-fixed, paraffin embedded sections (5 µm) on a Ventana Discovery Ultra autostainer. Following dewaxing and rehydration on board, epitope retrieval is performed using Ventana Ultra CC1 buffer at 96^{o}C for 64 minutes. Primary antibody is applied at 36^{o}C for 60 minutes. Primary antibodies are detected using an anti-rabbit HQ detection system followed by Chromomap DAB IHC detection kit, counterstaining with Mayer's hematoxylin, dehydration and mounting.

Imaging

17 The slides are scanned in Leica DM6 B upright microscope combined with an Aperio Versa 200 scanning system. 20X magnification has been used.