<section|Indirect Immunofluorescence>

1. Perform {fixation|operation} with {4%|concentration} of {para-Formaldehyde|solution} in {Phosphate-buffered saline (PBS)|buffer solution} with {1x|dilution} for {10|minutes}.
2. <for|repetitions|[1-3]|+|1>[[1]](#footnote-1) perform {wash|operation} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
3. Perform {permeabilization|operation} using {0.25%|concentration} of {Triton X-100|surfactant} in {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}

for {3| minutes}.

1. <for|repetitions|[1-3]|+|1>[[2]](#footnote-2) perform {wash|operation} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
2. Perform {45|minutes} of {incubation|operation} with {primary|antibody} .
3. Perform {three|repetitions} of {wash|operation} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
4. <for|repetitions|[1-3]|+|1>[[3]](#footnote-3) perform {wash|operation} each for {5|minutes} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
5. Perform {45|minutes} of {incubation|operation} with {secondary (fluorescent)|antibody (mix)} .
6. <for|repetitions|[1-3]|+|1>[[4]](#footnote-4) perform {wash|operation} each for {5|minutes} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
7. Perform {10|minutes} of {incubation|operation} with {DAPI)|fluorescent}.
8. <for|repetitions|[1-3]|+|1>[[5]](#footnote-5) perform {wash|operation} each for {5|minutes} using {1x|dilution} of {Phosphate-buffered saline (PBS)|buffer solution}.
9. Perform {mounting|operation} on a slide using {ProLong Gold|mountant} or {ProLong Diamond|mountant}.
10. Perform {polymerization|operation} on mounting medium at {room|temperature} RT over night.

**Primary antibodies:**

|  |  |
| --- | --- |
| **Mouse:** | |
| Monoclonal Anti-CENPA | 1:50 |
| Monoclonal Anti-Giantin | 1:100 |
| Monoclonal Anti-H2AX | 1:50 |
| monoclonal Anti-Lamin A | 1:100 |
| monoclonal Anti-Nup 153 | 1:100 |
| monoclonal Anti-Tom20 | 1:50 |
| **Rabbit:** | |
| Monoclonal Anti-CENPB | 1:50 |
| Monoclonal Anti-Clathrin | 1:100 |
| Monoclonal Anti-HP1 | 1:50 |
| monoclonal Anti-Ki67 | 1:100 |
| Monoclonal Anti-Nup133 | 1:50 |
| monoclonal Anti-Vimentin | 1:200 |

**Suggestions:**

**Mix 1:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Tom20 | 1:50 |
| Rabbit monoclonal Anti-Clathrin | 1:100 |

**Mix 2:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Lamin A | 1:100 |
| Rabbit monoclonal Anti-Vimentin | 1:200 |

**Mix 3:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Nup 153 | 1:100 |
| Rabbit monoclonal Anti-Vimentin | 1:200 |

**Mix 4:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Tom20 | 1:50 |
| Rabbit monoclonal Anti-Vimentin | 1:200 |

**Mix 5:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-CENPA | 1:50 |
| Rabbit monoclonal Anti-CENPB | 1:50 |

**Mix 6:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Lamin A | 1:100 |
| Rabbit monoclonal Anti-Ki67 | 1:100 |

**Mix 7:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Nup 153 | 1:100 |
| Rabbit monoclonal Anti-Ki67 | 1:100 |

**Mix 8:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-Nup 153 | 1:100 |
| Rabbit monoclonal Anti-Nup 133 | 1:50 |

**Mix 9:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti-H2AX | 1:50 |
| Rabbit monoclonal Anti- Ki67 | 1:100 |

**Mix 10:**

|  |  |
| --- | --- |
| Mouse monoclonal Anti- Nup 153 | 1:100 |
| Rabbit monoclonal Anti- Nup 98 | 1:50 |

1. <for|key|[range]|iteration\_operation|magnitude> [↑](#footnote-ref-1)
2. <for|key|[range]|iteration\_operation|magnitude> [↑](#footnote-ref-2)
3. <for|key|[range]|iteration\_operation|magnitude> [↑](#footnote-ref-3)
4. <for|key|[range]|iteration\_operation|magnitude> [↑](#footnote-ref-4)
5. <for|key|[range]|iteration\_operation|magnitude> [↑](#footnote-ref-5)