**Indirect Immunofluorescence:**

Fixation with 4% para-Formaldehyde in 1x PBS (10 min)

Wash 3x with 1x PBS

Permeabilization with 0.25 % Triton X-100 in 1x PBS (3 min)

Wash 3x with 1x PBS

Incubate with primary antibody mix (45 min)

Wash with 1x PBS (3x 5 min)

Incubate with secondary (fluorescent) antibody mix (45 min)

Wash with 1x PBS (3x 5 min)

Incubate with DAPI (10 min)

Wash with 1x PBS (3x 5 min)

Mount on a slide with ProLong Gold/ ProLong Diamond

Polymerization of mounting medium at 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 |

**Secondary antibodies:**

|  |  |
| --- | --- |
| αmouse-Alexa488 | 1:200 |
| αrabbit-Alexa488 | 1:200 |
| αmouse-StarGREEN | 1:100 |
| αmouse-Alexa568 | 1:200 |
| αrabbit-Alexa568 | 1:200 |
| αmouse-AbberiorSTAR580 | 1:100 |
| αrabbit-AbberiorSTAR580 | 1:100 |
| αmouse-AbberiorSTAR635P | 1:100 |
| αrabbit-AbberiorSTAR635P | 1:100 |
| αrabbit-StarRED | 1:100 |

**Fluorescently labeled F-actin probe (incubate together with 2. antibody):**

|  |  |
| --- | --- |
| Phalloidin-Atto488 | 1:100 |
| Phalloidin-Atto647N | 1:100 |

**Mix A:**

|  |  |
| --- | --- |
| αmouse-Alexa488 | 1:200 |
| αrabbit-AbberiorSTAR635P | 1:100 |

**Mix B:**

|  |  |
| --- | --- |
| αmouse-Alexa488 | 1:200 |
| αrabbit-Alexa568 | 1:200 |

**Mix C:**

|  |  |
| --- | --- |
| αmouse-Alexa568 | 1:200 |
| αrabbit-AbberiorSTAR635P | 1:100 |

**Mix D:**

|  |  |
| --- | --- |
| αmouse-AbberiorSTAR580 | 1:100 |
| αrabbit-AbberiorSTAR635P | 1:100 |

**Mix F:**

|  |  |
| --- | --- |
| αrabbit-Alexa488 | 1:200 |
| αmouse-AbberiorSTAR635P | 1:100 |

**Mix G:**

|  |  |
| --- | --- |
| αrabbit-Alexa488 | 1:200 |
| αmouse-Alexa568 | 1:200 |

**Mix H:**

|  |  |
| --- | --- |
| αrabbit-Alexa568 | 1:200 |
| αmouse-AbberiorSTAR635P | 1:100 |

**Mix I:**

|  |  |
| --- | --- |
| αrabbit-AbberiorSTAR580 | 1:100 |
| αmouse-AbberiorSTAR635P | 1:100 |

**Mix E:**

|  |  |
| --- | --- |
| αmouse-StarGREEN | 1:100 |
| αrabbit-StarRED | 1:100 |

**DNA-stain:**

|  |  |
| --- | --- |
| DAPI | 1:1000 |

**Your combinations:**

**! 2 with DAPI and 2 without DAPI**

**! At least one combination should be stained with Mix E (StarRED / StarGreen) secondary antibodies**

A B

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_