**Live-Cell Imaging CENP-A/Centrin in Cells**

****

DMEM (cat# 11995-040 / ThermoFisher)

DMEM/F12 1:1 Glutamax (cat# 10565-018 / ThermoFisher)

Leibovitz’s L-15, no phenol red (cat# 21083027 / ThermoFisher)

Fetal Bovine Serum (FBS) (cat# 7678 / Sigma)

Tet-Free FBS (cat# 631107 / Takara Bio)

L-Glutamine (Q) (cat# 25030-081 / ThermoFisher)

coverslips (cat# 12-545-81 / Fisher)

DMSO (cat# D2650 / Sigma)

Blasticidin (cat# ANT-BL-1 / InvivoGen)

Hygromycin B (cat# 10687010 / Invitrogen)

Puromycin (cat# P8833 / Sigma)

Protocol:

1. Plate cells on 22 x 22mm acid washed, #1.5 coverglass in a 35mm well 2-3 days prior to imaging
2. Assemble Rose Chamber in L-15 + 10% FBS + treatment [0.1% DMSO or 10 µM spastazoline] +
3. Allow chamber to incubate for 10-30 min at 35-37ºC while screening for cells on the Nikon Ti2-E microscope:
   1. Screen for metaphase cells using brightfield and add to XY multipoint list in Elements (v.5.02.00)
   2. Check cells from list for appropriate fluorescence signal using snapshots with laser light
4. Image as per Imaging Details

spastazoline (20200106 batch)

(382.52g/mol)

Cells:

Parental:

Designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Relevant Plasmid(s):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Generation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Thawed:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mycoplasma Free: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sequencing: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cell Type Verified: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Growth Conditions:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

Imaging Details:

Filename: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Objective: 100x 1.45 NA Plan Apo without Wollaston prism

Imaging Mode: Yokogawa W1 spinning disk

Illumination: 488 nm 100 mW laser (3%) 20 ms exposure

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

Camera: Prime95B sCMOS

z-step: 0.5 µm x 31 steps (15 µm volume) (PFS on)

Time: 30 sec interval, variable duration

**Live-Cell Imaging CENP-A/Centrin in Cells**

****

DMEM (cat# 11995-040 / ThermoFisher)

DMEM/F12 1:1 Glutamax (cat# 10565-018 / ThermoFisher)

Leibovitz’s L-15, no phenol red (cat# 21083027 / ThermoFisher)

Fetal Bovine Serum (FBS) (cat# 7678 / Sigma)

Tet-Free FBS (cat# 631107 / Takara Bio)

L-Glutamine (Q) (cat# 25030-081 / ThermoFisher)

coverslips (cat# 12-545-81 / Fisher)

DMSO (cat# D2650 / Sigma)

Blasticidin (cat# ANT-BL-1 / InvivoGen)

Hygromycin B (cat# 10687010 / Invitrogen)

Puromycin (cat# P8833 / Sigma)

Protocol:

1. Plate cells on 22 x 22mm acid washed, #1.5 coverglass in a 35mm well 2-3 days prior to imaging
2. Assemble Rose Chamber in L-15 + 10% FBS + treatment [0.1% DMSO or 10 µM spastazoline] +
3. Allow chamber to incubate for 10-30 min at 35-37ºC while screening for cells on the Nikon Ti2-E microscope:
   1. Screen for metaphase cells using brightfield and add to XY multipoint list in Elements (v.5.02.00)
   2. Check cells from list for appropriate fluorescence signal using snapshots with laser light
4. Image as per Imaging Details

spastazoline (20200106 batch)

(382.52g/mol)

Cells:

Parental:

Designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Relevant Plasmid(s):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Generation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Thawed:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mycoplasma Free: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sequencing: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cell Type Verified: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Growth Conditions:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

Imaging Details:

Filename: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Objective: 100x 1.45 NA Plan Apo without Wollaston prism

Imaging Mode: Yokogawa W1 spinning disk

Illumination: 488 nm 100 mW laser (3%) 20 ms exposure

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

Camera: Prime95B sCMOS

z-step: 0.5 µm x 31 steps (15 µm volume) (PFS on)

Time: 30 sec interval, variable duration

****

**Live-Cell Imaging CENP-A/Centrin in Cells**

DMEM (cat# 11995-040 / ThermoFisher)

DMEM/F12 1:1 Glutamax (cat# 10565-018 / ThermoFisher)

Leibovitz’s L-15, no phenol red (cat# 21083027 / ThermoFisher)

Fetal Bovine Serum (FBS) (cat# 7678 / Sigma)

Tet-Free FBS (cat# 631107 / Takara Bio)

L-Glutamine (Q) (cat# 25030-081 / ThermoFisher)

coverslips (cat# 12-545-81 / Fisher)

DMSO (cat# D2650 / Sigma)

Blasticidin (cat# ANT-BL-1 / InvivoGen)

Hygromycin B (cat# 10687010 / Invitrogen)

Puromycin (cat# P8833 / Sigma)

Protocol:

1. Plate cells on 22 x 22mm acid washed, #1.5 coverglass in a 35mm well 2-3 days prior to imaging
2. Assemble Rose Chamber in L-15 + 10% FBS + treatment [0.1% DMSO or 10 µM spastazoline] +
3. Allow chamber to incubate for 10-30 min at 35-37ºC while screening for cells on the Nikon Ti2-E microscope:
   1. Screen for metaphase cells using brightfield and add to XY multipoint list in Elements (v.5.02.00)
   2. Check cells from list for appropriate fluorescence signal using snapshots with laser light
4. Image as per Imaging Details

spastazoline (20200106 batch)

(382.52g/mol)

Cells:

Parental:

Designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Relevant Plasmid(s):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Generation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Thawed:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mycoplasma Free: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sequencing: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cell Type Verified: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Growth Conditions:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

Imaging Details:

Filename: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Objective: 100x 1.45 NA Plan Apo without Wollaston prism

Imaging Mode: Yokogawa W1 spinning disk

Illumination: 488 nm 100 mW laser (3%) 20 ms exposure

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

Camera: Prime95B sCMOS

z-step: 0.5 µm x 31 steps (15 µm volume) (PFS on)

Time: 30 sec interval, variable duration