V.2.2

Anti-SUMO-2/3 Mouse Monoclonal Antibody

Cat. # ASM24

Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Online Datasheet Contains
ASM24 (V2.2) and ASM24-S (V2.2)

Form:	Lyophilized powder
Amount of material:	2 x 200 µl when reconstituted
Validated applications:	IF and IP
Species reactivity:	Broad reactivity
Host/Isotype:	Mouse/IgG1-kappa
Clone:	11G2

Background Information

Small Ubiquitin-related Modifiers (SUMOs) are 12 kDa post translational modification (PTM) proteins that are highly conserved from yeast to mammalian cells[1]. In budding yeast (*Saccharomyces cerevisiae*), only one SUMO protein (Smt3) exists, in vertebrates, three major SUMO isoforms (SUMO-1, SUMO-2, and SUMO-3) are expressed in all tissue[2]. SUMO-1 is also known as SMT3C, Sentrin, GMP1, UBL1 and PIC1. Mature SUMO-2 and SUMO-3 are 97% identical in amino acid sequence (48% identity with SUMO-1) and appear to be functionally identical. SUMO proteins (SUMO-1 vs SUMO-2/3) show distinct sub-cellular localization, also the expression level of SUMO-2/3 is generally higher than that of SUMO-1[3-5]. Proteins are post translationally modified by SUMO conjugation (SUMOylation) to an acceptor lysine residue within a target protein consensus sequence ψ**K**XE (where ψ represents a hydrophobic amino acid and X represents any amino acid). While only a single SUMO-1 is ligated to target proteins, SUMO-2/3 proteins form poly-SUMO-2/3 chains on target proteins that can be terminated by SUMO-1 ligation[6,7]. SUMOylation is a highly dynamic reversible PTM that has been demonstrated to be involved in a diverse array of cellular processes, including regulation of gene expression, protein stability, protein transport, mitosis and protein-protein interaction[8,9].

Material

Anti-SUMO-2/3 antibody is a mouse monoclonal antibody. The antibody was raised against full-length recombinant SUMO-2 protein (Uniprot: P61956) combined with a proprietary mix of peptides that include CQIRFRFDGQPINE. The antibody has been shown to immunoprecipitate a wide range of SUMO-2/3 targeted proteins in a HeLa cell lysate (Fig. 1A). A linear epitope has not been identified and ASM24 appears to recognize a conformational epitope. Each Lot of antibody is quality controlled to provide a high batch to batch consistency. The Lot specific µg per tube can be found in the Lot specific COA documents at www.cytoskeleton.com. ASM24 is purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

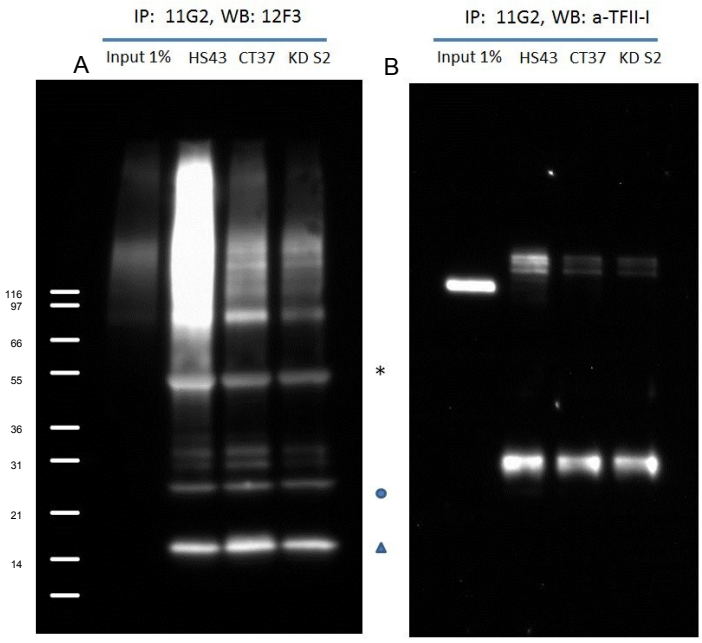
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder to the bottom of the tube. Reconstitute each tube in 200 µl of water and store at 4°C. DO NOT FREEZE. Final buffer composition is 200mM PIPES pH 7.4, 1% sucrose, and 0.5% dextran. When stored and reconstituted as described, the product is stable for 6 months at 4°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock.

Applications

Immunoprecipitation (IP) Applications

Working concentration should be determined by users empirically. IP performance of 11G2 has been confirmed using 30ul of 11G2 (Cat# ASM24) to IP SUMO 2/3 conjugated proteins and free SUMO 2/3 from 1mg of cell lysates.

Figure 1: IP of HeLa cells with ASM24 antibody



Legend: Denatured cell lysates were prepared from HS43, CT37 and KD S2[10] (HS43: Heat Shock treated (43°C for 10min), CT37: untreated and KD S2: shRNA SUMO-2 knock down). 1mg of lysate was used for the immunoprecipitation of SUMO-2/3 conjugates. IP experiments were performed by the protocol presented in IP and WB Method. Western blots of immunoprecipitated proteins were developed using anti-SUMO-2/3 (Cytoskeleon cat# ASM23) (A) or anti-TFII-I antibody (B). (A) Star (*) and circle (o) indicate heavy and light chains of antibodies. Unconjugated free SUMO is denoted by a triangle. (B) Unconjugated TFII-I is visible near 120kDa. Multiple bands indicate that TFII-I is SUMOylated by several SUMO-2/3 proteins. TFII-I has previously been reported to be a target for Sumoylation [10,11].

IP and WB Method

1. Incubate 30ul of 11G2 (Cat# ASM24) with 1mg of cell lysate (0.5mg/ml) for 1 hr at 4°C with rotation.
2. Add 30ul of Protein G slurry (in PBS) and incubate for 2 hr or overnight if convenient at 4°C with rotation.
3. Wash beads 3 times by resuspension in 1 ml of wash buffer (50mM Tris pH7.5, 150mM NaCl, 1% IGEPAL) followed by centrifugation (960 x g, 4°C, 1 min).
4. Resuspend beads in 30 µl of 2X non-reducing SDS sample buffer (125mM Tris pH6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue).
5. Incubate the solution at room temperature for 5min.

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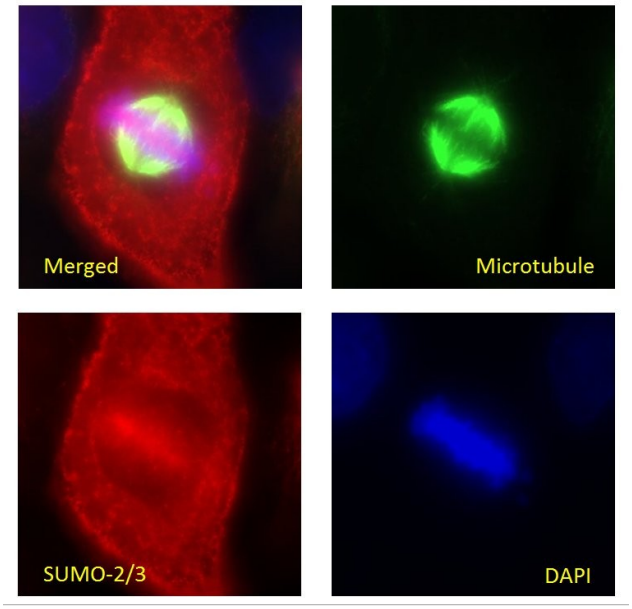
6. Pellet beads by centrifugation (960 x g, 4°C, 1 min) and collect the supernatant (~30ul) into fresh microfuge tubes.
7. Boil the sample with 1ul of beta mercaptoethanol for 5 min prior to loading on SDS -PAGE.
8. Run protein samples and control sample (we recommend 1% of IP input) in SDS-PAGE.
9. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
10. Transfer the protein to a PVDF membrane overnight (10-17h) at constant 20 V.
11. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
12. The membrane may be left in TBST overnight at 4°C if convenient.
13. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
14. Incubate the membrane with a primary antibody (e.g. For SUMO-2/3 use Cytoskeleton cat# ASM23) for 1-2 h at room temperature or overnight at 4°C with constant agitation. NOTE: we do not recommend clone 11G2 (Cat# ASM24) for western blot applications.
15. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
16. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-mouse secondary antibody (e.g., goat anti-mouse HRP conjugated IgG from Jackson Labs., Cat. # 115-035-068) in TBST for 60 min shaking at room temperature.
17. Wash the membrane 4 times in TBST, 10 min per wash.
18. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Immunofluorescence (IF) Applications

Use as indicated below at 1:500 dilution, sufficient for 200 ml of working strength Ab, approx. 200 IF applications.

1) Mitotic cell staining

Figure 2: IF of HeLa cells in metaphase with ASM24 antibody



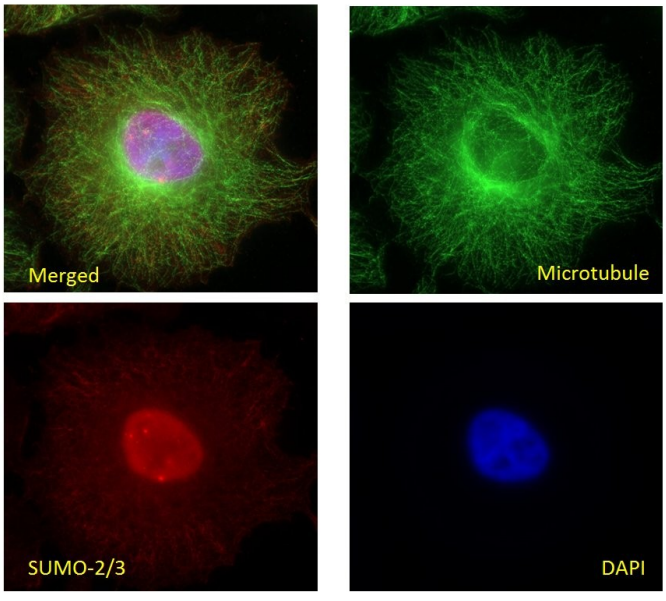
Legend: HeLa cells were stained and visualized by widefield fluorescence microscopy as described in the IF method below. The cells were stained against α/β-tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (11G2, red). DNA was stained with DAPI. Mitotic cells in metaphase were imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). The localization of SUMO 2/3-conjugates at chromosomes can be observed during mitosis as has been previously reported[12].

IF Method for mitotic cells

1. Plate HeLa cells at 3 x 10^5/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluency.
3. Permeabilize cells by incubating them with 3ml of digitonin solution (20mM HEPES pH 7.4, 110mM Potassium acetate, 2mM Magnesium acetate, 1X protease inhibitor cocktail, 10mM NEM and 40ug/mL digitonin) for 1 min with gentle agitation at room temperature. NOTE: we have found the timing of digitonin treatment and gentle agitation to be critical to successful chromosomal localization of SUMO2/3.
4. Wash the cell plate with PBS briefly (2 minutes incubation, no agitation).
5. Add 3ml of 4% paraformaldehyde solution and incubate for 10min at room temperature.
6. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
7. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
8. Apply 1ml of 11G2 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
9. Incubate at room temperature for 45 min.
10. Wash the cell plate two times with PBS (10 min. Incubation per wash, no agitation.
11. Apply 1ml of fluorescently-labeled secondary antibody solution at manufacturer's recommended dilution. For example, we use fluorescently-labeled donkey anti-mouse at 1:500 dilution in PBS to visualize SUMO-2/3 (red) and donkey anti-sheep at 1:500 to visualize microtubules (green).
12. Incubate at room temperature for 45 min.
13. Wash the cell plate two times with PBS with 10 min. incubation per wash.
14. Observe cells directly under inverted fluorescence microscope. NOTE: For observation of mitotic cells, we recommend direct observation without mounting. We have observed chromosomal localization with mounted cells using an upright microscope, however, the image quality is superior when using non-mounted cells in our hands.

2) Non-Mitotic cell staining

Figure 3: IF of HeLa cells in interphase with 11G2 antibody



Legend: HeLa cells were stained and visualized by widefield fluorescence microscopy as described in the IF method below. The cells were stained against β-tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (11G2, red). DNA was stained with DAPI. Cells in interphase were imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). PML nuclear bodies (nuclear dots) were visible in SUMO-2/3 staining as has been previously reported[5].

IF Method for non-mitotic cells

1. Plate HeLa cells at 3 x 10^5/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluency.
3. Fix cells by incubating the cells in 3ml of 4% paraformaldehyde solution for 10min at room temperature.
4. Wash the cell plate with PBS briefly (2 minutes incubation, no agitation).
5. Permeabilize cells by incubating in 3ml of 0.5% Triton X-100 solution for 5 min at room temperature.
6. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
7. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
8. Apply 1ml of 11G2 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
9. Incubate at room temperature for 45 min.
10. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
11. Apply 1ml of fluorescently-labeled secondary antibody solution at manufacturer's recommended dilution. For example, we use fluorescently-labeled donkey anti-mouse at 1:500 dilution in PBS to visualize SUMO-2/3 (red) and donkey anti-sheep at 1:500 to visualize microtubules (green).
12. Incubate at room temperature for 45 min.
13. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
14. Observe cells under fluorescence microscope.

References

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Product Citations/Related Products

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V.2.2

Anti-SUMO-2/3 Mouse Monoclonal Antibody

Cat. # ASM24-S

Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 150 µl when reconstituted
Validated applications:	IF and IP
Species reactivity:	Broad reactivity
Host/Isotype:	Mouse/IgG1-kappa
Clone:	11G2

Background Information

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Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder to the bottom of the tube. Reconstitute each tube in 150 µl of water and store at 4°C. DO NOT FREEZE. Final buffer composition is 200mM PIPES pH 7.4, 1% sucrose, and 0.5% dextran. When stored and reconstituted as described, the product is stable for 6 months at 4°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock.

Applications

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Working concentration should be determined by users empirically. IP performance of 11G2 has been confirmed using 30ul of 11G2 (Cat# ASM24) to IP SUMO 2/3 conjugated proteins and free SUMO 2/3 from 1mg of cell lysates.

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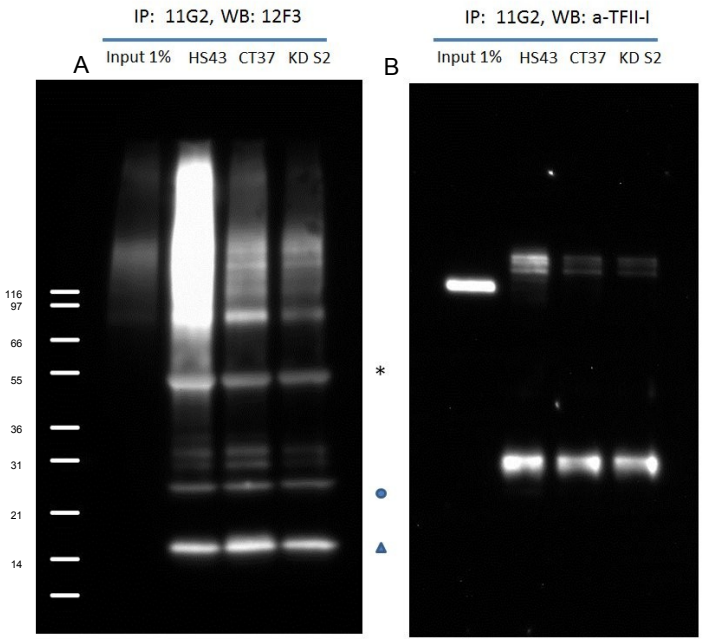

Legend: Denatured cell lysates were prepared from HS43, CT37 and KD S2[10] (HS43: Heat Shock treated (43°C for 10min), CT37: untreated and KD S2: shRNA SUMO-2 knock down). 1mg of lysate was used for the immunoprecipitation of SUMO-2/3 conjugates. IP experiments were performed by the protocol presented in IP and WB Method. Western blots of immunoprecipitated proteins were developed using anti-SUMO-2/3 (Cytoskeleon cat# ASM23) (A) or anti-TFII-I antibody (B). (A) Star (*) and circle (o) indicate heavy and light chains of antibodies. Unconjugated free SUMO is denoted by a triangle. (B) Unconjugated TFII-I is visible near 120kDa. Multiple bands indicate that TFII-I is SUMOylated by several SUMO-2/3 proteins. TFII-I has previously been reported to be a target for Sumoylation [10,11].

IP and WB Method

1. Incubate 30ul of 11G2 (Cat# ASM24) with 1mg of cell lysate (0.5mg/ml) for 1 hr at 4°C with rotation.
2. Add 30ul of Protein G slurry (in PBS) and incubate for 2 hr or overnight if convenient at 4°C with rotation.
3. Wash beads 3 times by resuspension in 1 ml of wash buffer (50mM Tris pH7.5, 150mM NaCl, 1% IGEPAL) followed by centrifugation (960 x g, 4°C, 1 min).
4. Resuspend beads in 30 µl of 2X non-reducing SDS sample buffer (125mM Tris pH6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue).
5. Incubate the solution at room temperature for 5min.

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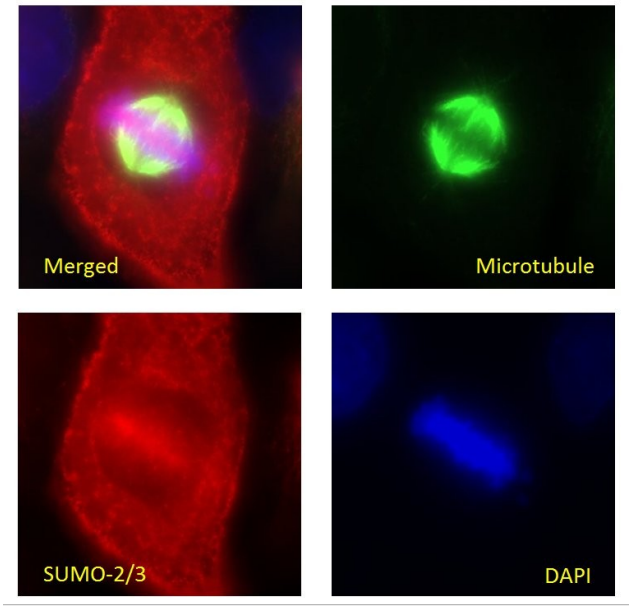
6. Pellet beads by centrifugation (960 x g, 4°C, 1 min) and collect the supernatant (~30ul) into fresh microfuge tubes.
7. Boil the sample with 1ul of beta mercaptoethanol for 5 min prior to loading on SDS -PAGE.
8. Run protein samples and control sample (we recommend 1% of IP input) in SDS-PAGE.
9. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
10. Transfer the protein to a PVDF membrane overnight (10-17h) at constant 20 V.
11. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
12. The membrane may be left in TBST overnight at 4°C if convenient.
13. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
14. Incubate the membrane with a primary antibody (e.g. For SUMO-2/3 use Cytoskeleton cat# ASM23) for 1-2 h at room temperature or overnight at 4°C with constant agitation. NOTE: we do not recommend clone 11G2 (Cat# ASM24) for western blot applications.
15. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
16. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-mouse secondary antibody (e.g., goat anti-mouse HRP conjugated IgG from Jackson Labs., Cat. # 115-035-068) in TBST for 60 min shaking at room temperature.
17. Wash the membrane 4 times in TBST, 10 min per wash.
18. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Immunofluorescence (IF) Applications

Use as indicated below at 1:500 dilution, sufficient for 75 ml of working strength Ab, approx. 75 IF applications.

1) Mitotic cell staining

Figure 2: IF of HeLa cells in metaphase with ASM24 antibody



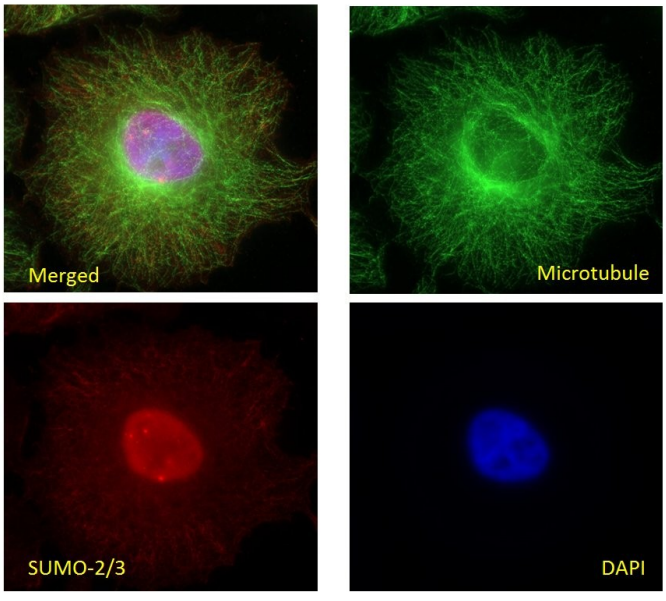
Legend: HeLa cells were stained and visualized by widefield fluorescence microscopy as described in the IF method below. The cells were stained against α/β-tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (11G2, red). DNA was stained with DAPI. Mitotic cells in metaphase were imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). The localization of SUMO 2/3-conjugates at chromosomes can be observed during mitosis as has been previously reported[12].

IF Method for mitotic cells

1. Plate HeLa cells at 3 x 10^5/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluency.
3. Permeabilize cells by incubating them with 3ml of digitonin solution (20mM HEPES pH 7.4, 110mM Potassium acetate, 2mM Magnesium acetate, 1X protease inhibitor cocktail, 10mM NEM and 40ug/mL digitonin) for 1 min with gentle agitation at room temperature. NOTE: we have found the timing of digitonin treatment and gentle agitation to be critical to successful chromosomal localization of SUMO2/3.
4. Wash the cell plate with PBS briefly (2 minutes incubation, no agitation).
5. Add 3ml of 4% paraformaldehyde solution and incubate for 10min at room temperature.
6. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
7. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
8. Apply 1ml of 11G2 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
9. Incubate at room temperature for 45 min.
10. Wash the cell plate two times with PBS (10 min. Incubation per wash, no agitation.
11. Apply 1ml of fluorescently-labeled secondary antibody solution at manufacturer's recommended dilution. For example, we use fluorescently-labeled donkey anti-mouse at 1:500 dilution in PBS to visualize SUMO-2/3 (red) and donkey anti-sheep at 1:500 to visualize microtubules (green).
12. Incubate at room temperature for 45 min.
13. Wash the cell plate two times with PBS with 10 min. incubation per wash.
14. Observe cells directly under inverted fluorescence microscope. NOTE: For observation of mitotic cells, we recommend direct observation without mounting. We have observed chromosomal localization with mounted cells using an upright microscope, however, the image quality is superior when using non-mounted cells in our hands.

2) Non-Mitotic cell staining

Figure 3: IF of HeLa cells in interphase with 11G2 antibody



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IF Method for non-mitotic cells

1. Plate HeLa cells at 3 x 10^5/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluency.
3. Fix cells by incubating the cells in 3ml of 4% paraformaldehyde solution for 10min at room temperature.
4. Wash the cell plate with PBS briefly (2 minutes incubation, no agitation).
5. Permeabilize cells by incubating in 3ml of 0.5% Triton X-100 solution for 5 min at room temperature.
6. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
7. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
8. Apply 1ml of 11G2 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
9. Incubate at room temperature for 45 min.
10. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
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References

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