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Home » Protocol specific for LC3B Antibody (NB600-1384)

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Fluorescent Staining

1. After washing sample(s) in PBS, fix for 20 minutes at room temperature.
2. Wash sample(s) in PBS 3x 3 minutes, each.
3. Block sample(s) with 5% NGS (keep Triton X-100 at 0.1%) for 60 minutes at room temperature
4. Prepare primary anti-LC3B (NB 600-1384) solution during this time at a 1:5,000 dilution with 1%NGS (keep Triton X-100 at 0.1%).
5. Incubate sample(s) in the primary Ab overnight at 4C in a humidified container

Note: If thick tissue samples, Ab must be prepared in 0.5% Triton X-100/PBS

Day 2

1. Wash sample(s) in PBS 3x 5 minutes with wells on. Prepare Alexa-fluor antibodies (Goat anti-Rabbit IgG H+L) as secondary Ab with 1% NGS (keep Triton X-100 at 0.1%).
2. Incubate sample(s) in the appropriate secondary Ab for 1 hour in a dark container.
3. Rinse the sample(s) in PBS 4x 10 minutes, each.
4. Place one drop of Vectashield onto the slide and cover with a coverslip

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