

In vitro Cathepsin D and Cathepsin B activity assays

To assess Cat D activation within lysosomes, we incubated blastocysts with Bodipy-FL-pepstatin A, which binds only active Cat D (Chen et al., 2000). Bodipy-FL-pepstatin A is a fluorescence-tagged cathepsin D inhibitor that binds to the active site of cathepsin D when its active site is open under acid pH conditions. To analyze Cat B activity *in vitro*, we used MagicRed (MR)-Cat B, a cresyl-violet conjugated (Arg-Arg)₂ peptide, which fluoresces only after it is cleaved by Cat B in an acidic environment (Erwig et al., 2006). For these reasons, Bodipy-FL-pepstatin A and MR-Cat B can be used as indirect indicators of Cathepsin D and B enzyme activities. These dyes can be used for both live and fixed cell studies (Yang et al., 2009).

1. Cells were seeded onto 24-well plates containing cover glasses or onto 35-mm glass-bottom dishes for fixation or live imaging, respectively, and grown to 70-80 % confluency.
2. A final concentration of 1 µg/ml of Bodipy-FL-pepstatin A (Invitrogen, P12271) (Chen et al., 2000) or MR-Cat B (Immunochemistry Technology, #937) (1:260, as suggested by vendor) was loaded for 1 hr or 30 min, respectively at RT.
3. As a positive control, cover glasses were treated with ammonium chloride (NH₄Cl), to inhibit Cat D activity (final concentration of 20 mM for 3 hrs), or leupeptin, to inhibit Cat B activity (0.3 mM for 6 hrs), added directly to the medium prior to assay.
4. Following 4 % PFA fixation, cells were further immunolabeled with Cat D, Cat B, or LAMP1 antibody for 4 hrs then visualized with Alexa Fluor 488 conjugated secondary antibody.
5. Cover glasses were mounted and analysed by confocal microscopy.

References

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