UbiQ
targeting the ubiquitin system

UbiQ protocol P010 _ Live cell delivery of ubiquitin reagents by bead loading.

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Description: we would like to highlight a straightforward, mild and cost-effective method for the application of ubiquitin(-like) reagents *in live cell experiments*. Reported by Brik and co-workers,[1-4] glass beads are shown to enable the direct cytosolic delivery of proteins in a process termed *bead loading* (Figure 1). Here glass beads induce physical disruption to cells to promote intracellular protein delivery. Importantly, treated cells exhibit normal proliferation rate, emphasizing the low toxicity of this approach. If you are interested in live cell ubiquitin experiments, please let us know and we can supply the glass beads (UbiQ-367).

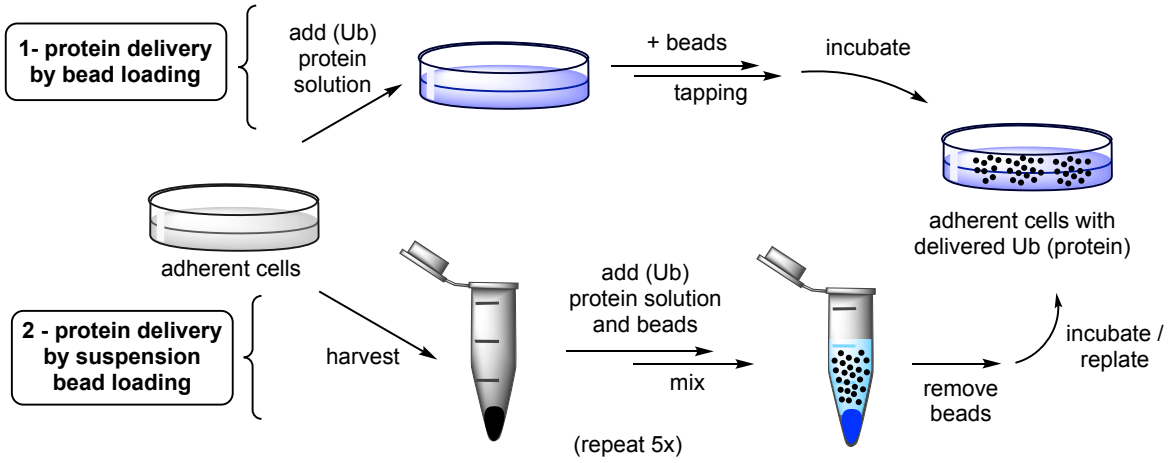

Figure 1. Intracellular protein delivery by glass bead loading.

Additional background can be found in references 1 – 4:

1. https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/cbic.202200122
2. https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.202300731
3. https://www.mdpi.com/1420-3049/27/15/4867
4. https://pubmed.ncbi.nlm.nih.gov/39246272/

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General procedure 1 - for protein delivery by bead loading

1. U2OS cells were cultured in PLL-treated removable chamber 8-well chamber slides (Ibidi, 80841) to 70% confluence.
2. a day before fixation, cells with interphase and mitotic cell cycle stages were enriched by incubation with hydroxyurea and colcemid, as described above.
3. after 12 h of incubation with cells cycle inhibitors, TAMRA-Ub (UbiQ-003) was diluted 1000x from a 5 mM DMSO stock (45 mg/mL) into sterile PBS containing 0.1% pluronic® F-68 (24040032, Gibco) to 5 µM.
4. next, the culture medium was removed, and cells were gently washed once with warm PBS + 0.1% pluronic® F-68 solution.
5. to the washed cells, 150 uL of the TAMRA-Ub solution was added (to cover the cells) and a monolayer of sterile glass beads (≤106 µm, acid washed) was sprinkled over the cells.
6. the culture slide was tapped on a bench eight times, with gentle swirling of the slide for a total of two times in between tapping and incubated for 2 min at 37 °C under 5% CO2. Following incubation, the cells were gently washed with DMEM (0.3 mL × 2) and incubated at 37 °C under 5% CO2 with colcemid containing DMEM for 4 h.

General procedure 2 - protein delivery by suspension bead loading

1. detach cells from the flask and pellet them (3min at 2500xg)
2. resuspended cells in an eppendorf with PBS containing 0.1% Pluronic® F-68
3. pellet the cells using a mini spin centrifuge (3min at 1000xg) and dispose the supernatant
4. add 100 µL of protein solution (5 uM) followed by glass beads addition
5. mix the cells, protein solution and beads by pipetting, vortex for 5 seconds and centrifuge for 10 seconds.
6. Repeat step 5 three times
7. after the final cycle, remove the supernatant, wash the cells once with medium, and re-pellet
8. resuspend the pellet with fresh medium and keep it for 3 seconds to allow the beads to settle
9. the supernatant containing the cells can be transferred to a new eppendorf tube, where they can be pelleted, lysed or counted and seeded onto Ibidi culture slide for imaging.