**Depletion of mtDNA with Ethidium Bromide**

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**Abstract**

This protocol describes the depletion of mitochondrial DNA (mtDNA) from HeLa cells using ethidium bromide (EtBr).

**Keywords**

Mitochondrial DNA (mtDNA), ethidium bromide.

**Solutions to prepare**

**DMEM** containing 10% FBS and 2 mM L-glutamine (Gibco)

**DMEM** containing 10% FBS, 2 mM L-glutamine (Gibco), and 2 mg/mL ethidium bromide

**RIPA buffer:** 150 mM NaCl, 10 mM Tris, 0.5 mM EDTA, 0.5% NP40 supplemented immediately before use with Protease Inhibitor Cocktail (Roche) and PhosStop phosphatase inhibitor (Roche)

**Depletion of mtDNA in HeLa cells using EtBr**

1. Plate HeLa cells in DMEM in 6 well format (2 x 105 cells per plate).
2. Incubate 24 hours.
3. Aspirate DMEM and replace with DMEM containing 2 mg/mL ethidium bromide.
4. After two days, replace media with fresh DMEM containing 2 mg/mL ethidium bromide.
5. After two more days, passage cells into new 6 well plates in DMEM containing 2 mg/mL ethidium bromide.
6. After two more days, replace media with fresh DMEM containing 2 mg/mL ethidium bromide.
7. At day 8, lyse cells and collect total DNA using DNeasy kit (Qiagen), RNA with RNeasy kit (Qiagen), and protein in RIPA buffer (as described in Immunoblotting protocol).