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|  **Nuclear extraction**Last modified 11.01.2012 |
| **Materials** |
| * Phosphate buffered saline (PBS)
* Buffer A (low salt): 10mM HEPES-KOH pH 8.0, 1.5 mM MgCl2, 10 mM NaCl
* Buffer A\*: Buffer A + protease inhibitors + 0.15% NP40
* Buffer C (high salt): 20mM HEPES-KOH pH 8.0, 2 mM MgCl2, 420 mM NaCl, 20% (v/v) glycerol, 0.2 mM EDTA, 0.1% (v/v) NP40. **Add fresh before use: 0.5 mM DTT, complete protease inhibitors mini EDTA free (1 tablet/10 ml)** (Roche 11836170001)
* Fresh cells (not frozen pellets)
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| **Equipment** |
| * Glass douncer with type B pestle (in 500 µl, 2ml, 7 ml and 15 ml volumes)
* Cooled bench centrifuge (Heraeus Multifuge 3 S-R; for 15, 5 or 500 ml tubes)
* Cooled tabletop centrifuge (Heraeus Fresco 21; for 1.5 or 2 ml vials)
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| **Procedure** | **Comments** |
| **A) Harvesting cells (adherent cells)** |
| 1. Wash cells with 10 ml PBS
2. Trypsinise with 1.5 ml trypsin/15 cm dish
3. Resuspend with 10 plates with 10 ml of medium
4. Pool in a 50 ml tube
5. Repeats steps 3-4
6. *Perform all subsequent steps at 4 °C/on ice*
7. Centrifuge cells 5 min, 400 g, 4 °C. Remove supernatant
 | * Before starting, pre-cool buffer A, buffer A\*, buffer C , PBS and the douncer on ice
* For recommended douncer volumes, refer to step 24 comments
* These steps may vary; in general, wash, trypsinise, neutralise, resuspend and pool
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| 1. Resuspend cells with 50 ml PBS
2. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant
3. Repeat steps 8-9
4. Resuspend cells with 8 ml PBS and transfer to the 15 ml tube
5. Rinse the 50 ml tube again with 5 ml PBS and transfer to the 15 ml tube
6. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant
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| **B) Harvesting cells (suspension cells)** |
| 1. Centrifuge 500ml of cells in a 500ml tube for 5 min, 400 g, 4 °C. Remove supernatant
2. Resuspend cells with 50 ml PBS
3. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant
4. Repeat steps 14-15
5. Resuspend cells with 8 ml PBS and transfer to the 15 ml tube
6. Rinse the 50 ml tube again with 5 ml PBS and transfer to the 15 ml tube
7. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant
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| **Mechanical lysis of cells** |
| 1. Determine cell pellet volume and resuspend cells with 5 volumes of buffer A
2. Incubate on ice for 15 min
3. Centrifuge 5 min, 400 g, 4 °C. Remove supernatant
 | * **Cell pellet should swell after incubation** with buffer A. Increase in cell volume may vary depending on cell type.
* While waiting, wash douncer with demi water and rinse with buffer A.
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| 1. Determine cell pellet volume and resuspend in 2 volumes of **buffer A\***
2. Apply 3 x 10 to 4 x 10 strokes. Wait 30s after every set of strokes
3. Transfer the cell suspension to a 15 ml tube
4. An aliquot of cell suspension can be checked under a microscope to see whether the cells have lysed from douncing. Perform during step 28.
 | * **Recommended douncers**:
	+ 0.1 ml douncer: >50 µl swelled pellet
	+ 2 ml douncer: 50-600 µl swelled pellet
	+ 7 ml douncer: 600-2500 µl swelled pellet
* **The plunger must remain in the cell suspension** to avoid air bubbles
* The 30s rests between sets of strokes prevent heating up of the cell suspension that may result in protein instability
* Douncing opens up cells (but not nuclei)
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| 1. Centrifuge 15 min, 3200 g, 4 °C
2. Transfer or discard the supernatant (**cytoplasmic extract (CE)**)
 | * **To store the CE, add glycerol (10% final conc.) and NaCl (150 mM final conc.)**. If the nuclear extraction has priority perform after step 29
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| **Lysis of nuclei** |
| 1. Determine the volume of cell pellet (**crude nuclei**)
2. Wash the pellet with 10 volumes of PBS. **The nuclei are very fragile. Quickly add the PBS and invert. Do not pipette up and down.**
3. Centrifuge 5 min, 3200 g, 4 °C. Discard supernatant
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| 1. Determine the volume of cell pellet
2. Add 2 volumes of buffer C
3. **Cut the tip of a 1 ml pipette and resuspend as best as possible by pipetting up and down vigorously**
4. Transfer the suspension to 1.5 or 2 ml vials
5. Incubate 1 h, 4 °C on a rotating wheel
 | * After incubation, the suspension will become viscous and white clouds of chromatin should appear
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| 1. Centrifuge on a table-top centrifuge for 45 min, 20,800 g or max speed, 4 °C
2. Aliquot the supernatant (**nuclear extract (NE)**) and snap freeze with liquid nitrogen
3. Store at -80 °C
 | * An aliquot of NE can be analysed for protein conc. via Nanodrop. Expected conc.: ~5 mg/ml
* The pellet contains chromatin
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