Hi, I'm having some problems with making nuclear extract from NK92 cells (lymphocytes, suspension). The problem is that after I collect nuclei and try to resuspend in my nuclear extract buffer, I cannot get it to resuspend and the prep turns clumpy and gooey. I suspect I've busted the nuclei and released genomic DNA, but there is no detergent in extraction buffer so I'm not sure how it's possible. Here's my protocol

1. wash ~1x10^6-1x10^7 cells x2 in cold PBS
2. pellet cells 5k rpm 5min 4C
3. Lyse cells using 500ul of lysis buffer (Tris/EDTA, 60mM KCl, 1mM DTT, protease inhibitor tablet, 0.1% NP-40) for 5 min on ice
4. Pellet nuclei at 2.5k rpm 4 min 4C
5. Wash with buffer (same as lysis buffer but no NP-40) once - still not clumpy, easily resuspended at this point
6. pellet nuclei at 2.5k rpm 4 min 4C
7. Resuspend in 100ul nuclear extract buffer A (Tris/EDTA, 420mM NaCl, 1.5mM MgCl2, 25% glycerol) OR buffer B (same as A, but with 800mM NaCl instead of 420mM) - after addition, pellet becomes clumpy and gooey
8. ice for 20 min, spin down at max speed 6min 4C and take supernatant

Also, when I take the supernatant, I end up with less than 100ul. I think I'm losing a fraction to being trapped in the clump/goo.

Any insights much appreciated...

Ben

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