24/06/21 - Rep 1 IP

→ old mouse (w/ lots of fluid and femurs bones) + young mouse

→ Bones were crushed thoroughly 6x 5 mL w/ Euglicis (RT)
→ 10min RT, Filled w/ PBS-only!
→ spindown 5min, 500×g, 4°C
→ Resuspended in 800µl PBS-only and transfered to low-binding
→ spindown AGD resuspended in TRIS lysis buffer: 1 mL
  . prepared w/ MS grade buffers (All but 10% NP-40) and filtered
  . thoroughly vortex until all was sol. and a huge DNA pellet was seen
→ spindown 17K×g, 15min, 4°C
→ sup was collected
→ 5µl was use w/ 45µl PBS w/ 660 nm for protein concentration:

y ⇒ 2.9 mg/µl
o ⇒ 3.9 mg/µl

⇒ IP: will respect the 1mg input : 5µl bead slurry ratio and scale IP reaction accordingly.

y (2.9 mg) per IP → 1.45 mg
- α Nuc1 (500µl) + 7.25µl beads
- α IgG (500µl) + 7.25 beads

o (3.9 mg) per IP → 1.95
- α Nuc1 (500µl) + 7.25µl beads
- α IgG (500µl) + 7.25 beads

- beads were added to new eppies and wash 3x w/ lysis buffer
- IP was incubated 30', rotator, 4°C

⇒ samples were washed 3 x 1 ml lysis buffer:
- second wash changed eppies

⇒ After last wash extra spin to remove left over
⇒ Add 25µl 2% SDS 40mM Tris as elution buffer
⇒ 5 min, 70°C, 1000 rpm
⇒ collect volume in new eppi and store -20°C

06/01/2021 - Rep #2

- 1 young + 1 old (both from Janvier) looking good

→ brains were cleaned and crushed 6x 5ml w/ lysis buffer
  - Ellen did the young one
→ lysis 10min, RT, spindown
→ Resuspend in 800µl PBS only
→ transfered to low-binding eppie, spun down
→ y/o samples were resuspended in 1ml tris buffer + PI
→ spindown 15 min, 4°C, 17k xg
→ 5µl for 660nm quant:

young ⇒ 453.2529 µg/ml ×10 = 4.53 mg/ml (0.453 mg)

old ⇒ 460.6634 µg/ml ×10 = 4.60 mg/ml (0.460 mg)

IF 1mg - 5µl
4.6mg - (23µl) use 23µl bead slurry of αMuOl / αIgG

- was 23µl beads 3x 1ml Tris buffer
- there was not enough ACN vol so for old was taken from 7 eppie but still same batch.
- 500µl of lysate was added to beads and resuspended
- Rotator @20, 30min, 4°C
- sort spin down to bring bead down
- 3x 1ml washes w/ eppie change in #2
- extra spin to remove all buffer
- add 25µl 2% SDS 40mM Tris elution buffer
- resuspend beads in elution buffer
- incubate 5min, 70°C, 1000 rpm
- collect elution in new eppie store -20°C

extraction buffer was freshly prepared w/ normal recipie:
- ask leiba about her notes for #1

13/07/2021 - Rep #3

1 young + 1 old (~20 mo) from Janvier looking good.

- bones were collected in HBS/BSA
- crushed 6x 5min RT lysis ⇒ Ellen crushed spin one
- 10min RT
- spin down 5min 500xg 4°C
- transfered to low binding eppie
- spin 17K xg, 15min, 4°C
- sup was collected in new eppie
- 5µl of each sample was used for 660nm quant

young ⇒ 462.22396 µg/mL (0.46222 mg) ×10 ⇒ 4.62 mg/mL 23.1 µl beads (Add 23 µl)

old ⇒ 418.86679 µl/mL ×10 ⇒ 4.18 mg/mL 20.9 µl beads (Add 21 µl)

⇒ beads were washed 3x 1ml of TBS buffer

⇒ 500µl of cell lysate was added to IP eppie and vortexed

⇒ incubation @ 20, 30min, 4°C

⇒ beads were washed again 3x 1ml buffer

· second was there was eppie change

⇒ 3S extra spin down after first wash to remove excess but.

⇒ 25µl 2% SDS 40mM Tris pH 8.0

⇒ 70°C, 5min, 1000 rpm

⇒ collect elution into new eppie