**Extract protein from tumor (Xenograft) tissue**

* Cryostat: cut 8 slices of 50 µm – keep on dry ice in Eppendorf
* Keep Eppendorf on ice
* Add 200 µL of RIPA + protease inhibitor (RIPA = 4°C, RIPA + PI =-20°C)
* Keep it for 30 min on ice
* Flash freeze 3 times (liquid nitrogen – 37°C)
* Centrifuge Eppendorfs for 15 min at high speed
* Transfer supernatant in a autoclaved Eppendorf

**Protein concentration (Bradford assay)**

Dilute Biorad protein assay dye reagent 5x (red, fridge weighing room) in MQ

* + For each standard/sample = 1 mL
  + Protect from light!

Add 1 mL to cuvet for each standard (5)/sample

Add standard or sample:

* + 0, 2, 4, 8 ,16 µL of BSA (1 mg/ml in MQ)
  + 5 µL of sample
  + Vortex the samples

Measure samples with spectrophotometer:

* + Use Bradford program
    - Measure blank
    - Measure each standard/sample (sample)
    - Use absorbance at A595

Use excel file: Bradford Protein Assay:

* Determine equation BSA standard curve
* Calculate concentrations of samples
  + Change formula
  + Adjust concentration loading (20 µg/blot) – how many times you want to do WB?
  + Adjust the total volume you want to have (e.g. 20 µg/25 µL = load 25 µL)

Dilute protein samples:

* … µL of sample
* … µL of 6x Laemlli + freshly add B-ME
* … µL of MQ

Vortex samples + cook at 95-100°C for 2 min (2 times)

Centrifuge your samples

🡪 Store at -20°C