



**iGEM Pasteur Paris 2016**  
*Protocols*



## CELLULOSE BINDING TEST

**Aim:** Verify that the C2 protein binds to cellulose.

**Protocol:**

- in a 2ml Eppendorf tube, put:
  - 10mg of Avicel cellulose
  - 1ml of the used protein purification buffer: PBS (150mM NaCl, 50mM sodium phosphate) or Buffer A (150mM NaCl, 50mM Tris, pH=7,4)
  - 10 $\mu$ l of BSA (1 $\mu$ g/ $\mu$ l)
  - 100 $\mu$ l of the fraction of the purified protein C2

*Use a mixture without C2 protein as a negative control.*

- incubate at room temperature for 1 hour, under stirring.
  - centrifuge the tube at 13,200rpm for 5min.
  - recover the supernatant and store it in a 1.5ml Eppendorf tube.
  - wash the pellet with the buffer used before (stir for 5min).
  - centrifuge the tube at 13,200rpm for 5min.
  - recover the supernatant and store it in another 1.5ml Eppendorf tube.
  - denaturate the pellet with 200 $\mu$ l of Laemli 2x at 95°C for 5min.
  - denaturate 10 $\mu$ l of the two previous supernatants with 10 $\mu$ l of Laemli 2x at 95°C for 5min.
  - analyse the three samples on an SDS PAGE protein gel.
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