ECL detection

Solutions

	ECL soloution I	**ECL souloution II**
250mM lumiol in DMSO	5ml	5ml
90 mM p-coumaric acid in DMSO	500ml	
30% H_2O_2	220ml	30.5 ml
Final	50ml	50ml

1. Incubate the membrane in a plastic container with 15ml PBS- TM; shake gently and ensure that the membrane is always covered with solution (30min)
2. Remove blocking solution
3. **Primary antibody**: Depending on the antibody titer and the abundance of the target protein, dilute primary antibody by 1:500 to 1:20.000 in 15ml PBS-TM and incubate the membrane with the solution for 1h with gentle shaking (We will use HA- antibody: 1:10.000
4. Discard the secondary antibody solution.
5. Rinse membrane twice with a little PBS-T and wash membrane 3x5 min with 15ml PBST
6. **Secondary antibody**: Dilute secondary antibody (usually goat anti-rabbitcoupled to horse radish peroxidase) at a 1:10.000 dilution in 15ml PBS-TM and incubate the membrane with this solution for 1h with gentle shaking.
7. Discard the secondary antibody solution.
8. Rinse membrane twice with a little PBS-T and wash 3x5 min with 15 ml TBS-T.
9. Cut Whatman paper to a size a little larger than the membrane and place it onto the FUSION detection tray.
10. For each mini-gel blot mix 1,5 ml of ECL solution I and 1,5 ml of ECL solution II in a test tube and vortex well (carful: do not cross- contaminate ECL solution, this will destroy them). **Work with gloves.**
11. Distribute the mixed ECL solutions evenly across the Whatman filter paper; squeeze out bubbles with a test tube.
12. Place membranes with the protein side up onto the Whatman filter paper.
13. Detect the chemiluminescence signal with the FUSION device.

Screen for protein secretion

This protocol is for testing the supernatant of our strains for secreted proteins. After this workflow, you should have an idea, which of your stains secrete the proteins and how strong the secretion is. If you identify a good candidate via this protocol, you calculate the amount of supernatant needed from that strain, so we can downscale the screening approach for a faster workflow.

Work on ice and pre cool all tubes.

- Dilute pre cultures to 2x10^5 in 50 ml TAP.

- Grow for 2 days to ca. 5-6 x 10^6 cells / ml (check for similar green colour)
- Harvest 10 ml in non- sterile 15ml falcons at 3800rpm for 2 min.

TCA- precipitation protocol:

- Transfer supernatant to fresh 15 ml falcon and freeze cell pellet, repeat centrifugation to remove residual cells from medium.
- Transfer supernatant to fresh 50ml falcon
- Add 10 ml 20% TCA solution to the falcons with supernatant for protein precipitation (20% w/v in H2O, CAUTION: TCA is a strong acid, wear gloves, goggles and handle with care!)
- Incubate supernatant + TCA for 30 min on ice
- Transfer supernatant + TCA to 40ml Avanti tubes, centrifuge 15 min at 20.000g, 4 C° (CAUTION: Balance tubes before centrifugation, high- speed centrifugation at these volumes can go horribly wrong!)
- Remove supernatant and 1x PBS 400µL of the supernatant to the respective Avanti tubes. Thoroughly resuspend the pellet and wash the bottom of the tube.
- Add 1,6 ml cold acetone to each 2 ml tube and spin for 5 min at full speed, 4 C° to precipitate the protein again.
- Remove supernatant and dry pelletunder the hood (takes about WELLE 30min)
- 5min 80 mbar in speed vac
- Resuspend protein pellets in 30 MIKROL 2xSDS loading buffer.
- 2min by 95 GRAD CELSIUS
- Load 15 MIKROL each on SDS gel, do western blot (The resembles the amount of protein in about 5 ml of your supernatant).

2xSDS loading buffer:

	Volumes	**Final concentration**
1M Tris pH 6.8	600MIKROL	60mM
Glycerol	5 ml	50%
20% SDS	1 ml	2%
Bromphenolblue	A really small tip of a spatula	Really not much
1M DTT	1 ml	100 mM
H2O	2.4 ml	-
Final	10 ml	-

	Volumes
0,5M Tris pH 6.8	120MIKROL
Glycerol	5 ml
20% SDS	1 ml
Bromphenolblue	A really small tip of a spatula
1M DTT	1 ml
H2O	1.8 ml
Final	10 ml

In case of yellow discoloration (caused by TCA residues) add NaOH in 1 MIKROL step until blue discoloration is reached.

Add DTT at the end, mis vigorously by vortexing and split up tu1mL aliquots, store at -20 GRAD CELSIUS. DTT degrades quickly at room temperature.