AHK4 Assay Protocol

Materials

E. coli KMI002 strain was used in the experiments. The KMI002 (ΔrcsC, *cps::lacZ*) strain lacks *rcsC* gene and habors *cps::lacZ* fusion gene.

A high-copy plasmid, pSB1C3, was used in the experiments and the *ahk4* gene was inserted downstream the BAD/*araC* promoter, an L-arabinose inducible promoter, followed by a ribosome binding site

Qualitative experiment

1. LB agar plates containing chloramphenicol (34 μg/mL) were prepared.
2. 50 μl of X-Gal (50 mg/ml), 10 μl of 100 mM iP or DMSO as a control, and 40 μl of LB medium was mixed in microtubes. Then the solutions were applied to the agar plates.
3. Samples were inoculated and incubated at room temperature.
4. Photographs were taken after sufficient blue color was developed.

Quantitative experiment

1. Overnight culture of samples were prepared in 2 ml of LB medium containing chloramphenicol (34 μg/mL) at 25℃.
2. Samples were diluted for 2000-fold in 1ml of fresh LB medium containing chloramphenicol (34 μg/mL) and various concentration of IP (10 nM-100 μM). Cells were also inoculated into medium containing DMSO instead of iP.
3. Samples were cultured overnight at 900 rpm at 25℃.
4. Cells were collected by centrifugation at 10,000 × g for 10min.
5. All of supernatant was discarded and then cells were resuspended in 500 μL of PBS buffer containing 1 mM $MgSO_4$ and 1 mM dithiothreitol (DTT). Also 500 μL of the same buffer in was prepared as a control for spontaneously splitting of ONPG.
6. 20 μL of each suspension was added into 180μL of the buffer used above and Abs600 was measured and recorded by a microplate reader.
7. 10μL of 0.1% SDS and 10 μL of chloroform was added into each tube including the control and vortexed for 15sec.
8. Tubes were heated at 28℃ for 5min.
9. 100 μL of ONPG (4 mg/mL) was added to each tube and incubated at 37℃ for 30min. ONPG was dissolved in the buffer used above.
10. After 30min incubation, tubes were heated at 65℃ for 10min to inactivate β-galactosidase.

11. All samples were centrifuged at 15,000 rpm for 10min.
12. Abs420 of supernatant was measured and recorded by a microplate reader. The control was used as a blank.
13. Relative β -galactosidase activity was calculated by following formula:

$$\text{Relative } \beta\text{-galactosidase activity} = \frac{Abs420}{Abs600 \cdot 10 \cdot 30min}$$