

## Cas13a Activity *in-vitro* Assay

- To perform this assay, the RNase Alert from Thermo Fischer was used.
- Resuspend RNase Alert in 5  $\mu\text{L}$  RNase Alert Buffer (5x) and 45 ml RNase free water.
- Target RNA isolation from overnight culture (Here: *E. coli* DH5 $\alpha$  with pSB1K3\_RFP, purified with RNA isolation kit from ZYMO Research)
- Pipetting scheme:

	Cas + target RNA + crRNA	Cas + target RNA	crRNA + target RNA	Cas + crRNA	Target RNA
<b>RNase Alert</b>	45 $\mu\text{L}$	45 $\mu\text{L}$	45 $\mu\text{L}$	45 $\mu\text{L}$	45 $\mu\text{L}$
<b>Cas13a reaction Buffer</b>	10 $\mu\text{L}$	10 $\mu\text{L}$	10 $\mu\text{L}$	10 $\mu\text{L}$	10 $\mu\text{L}$
<b>crRNA</b>	0.2 nM		0.2 nM	0.2 nM	
<b>Target RNA</b>	10 $\mu\text{L}$	10 $\mu\text{L}$	10 $\mu\text{L}$		10 $\mu\text{L}$
<b>Cas13a</b>	2.3 $\mu\text{M}$	2.3 $\mu\text{M}$		2.3 $\mu\text{M}$	
<b>water</b>	to 100 $\mu\text{L}$	to 100 $\mu\text{L}$	to 100 $\mu\text{L}$	to 100 $\mu\text{L}$	to 100 $\mu\text{L}$

- Measurement of fluorescence intensity with TECAN infinite M200, excitation: 490 nm, emission: 520 nm, every 5 min for 12 h. Gain: 75.

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