

In vitro Cleavage Assay using *Streptococcus pyogenes* Cas9 protein

1. Set up the reaction mixture as below. Instead of 10x Cas9 Reaction Buffer (Cat No CB01), NEB Buffer 3 and BSA can be used. Add target DNA the last for the best result.

Components	Amount	Range
Cas9 protein	150 ng	50~500 ng
sgRNA	100 ng	30~200 ng
Target DNA	60 ng PCR product	50~100 ng
	or 100 ng plasmid	50~200 ng
10x Cas9 Reaction Buffer (Cat No CB01)	1 ul	
Nuclease-free water	to 10 ul	

2. Incubate the reaction mixture at 37 °C for 30 minutes to 1 hour.
3. Heat to inactivate Cas9 protein at 65 °C for 10 minutes.
4. Analyze on an agarose gel.
5. Make sure to run the control sample (without Cas9 protein or without sgRNA) in parallel.