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# Product Information Sheet:
# Cas9, 10mg/mL*

## Product Description

**Cas9, 10mg/mL:**

Cas9 is an RNA-guided endonuclease that is naturally expressed by *Streptococcus pyogenes*. Catalytically active ribonucleoprotein (RNP) complexes are produced by incubating Cas9 with a sequence specific CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), which together activate the endonuclease and guide it to the ~20-nucleotide protospacer sequence in target dsDNA. Alternatively, Cas9 can be complexed with a synthetic guide RNA (sgRNA) that encodes both the crRNA and tracrRNA in a single RNA strand. Recombinant Cas9 includes a C-terminal nuclear localization signal (NLS) derived from the human nucleoplasm in gene to allow for import of RNP complexes into the nucleus of transfected mammalian cells. RNP complexes can be used for in vitro cleavage assays using the included reaction buffer or transfected into competent cells using electroporation. Properly targeted Cas9 RNPs will specifically cleave dsDNA within the target sequence and remain bound to the target strand until denaturation or proteolytic digestion of the enzyme.

**10X Cas9 Reaction Buffer:**

Specially optimized reaction buffer containing Sodium Chloride, Tris-HCl, Magnesium Chloride, and BSA at pH 7.9. This Buffer is supplied in a 10X concentration and should be diluted for use.

## Concentration

Cas9: 10mg/mL
Cas9 Reaction Buffer: 10X

## Storage and Handling

Store at -20°C upon arrival until provided expiration date. See individual component labels for additional storage recommendations. Minimize freeze / thaw cycles to avoid loss of performance. Maintain product on ice during use.

**Cas9 Storage Buffer:**

10mM Tris-HCl, 300mM NaCl, 1mM TCEP, 0.1mM EDTA, 50% Glycerol, pH 7.4 @ 25°C.

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## Protocol

1. Dilute Cas9 enzyme to 1 µM. Note: 0.16mg/mL is equal to 1uM of Cas9.
2. Assemble a 30 µl reaction using Table 1.

### Table 1: Reaction Master Mix

| Component | Volume | Final |
|---|---|---|
| Nuclease-free water to Volume | X µl | - |
| 10X Cas9 Reaction Buffer | 3.0 µl | 1X |
| 300nM sgRNA | 3 µl | 30 nM |
| 1uM Cas9 | 1 uL | 30 nM |
| Total | 27 µl | |
| Incubate for 10-minutes at 25°C | | |
| 30 nM substrate DNA | 3 uL | 3 nM |
| Total | 30 µl | |

3. Mix thoroughly. Spin the reaction down using a microcentrifuge, if necessary.
4. Incubate at 37°C for 15 minutes.
5. Add 1 µL of Proteinase K (20 mg/mL) to each sample. Mix thoroughly. Spin the reaction down using a microcentrifuge, if necessary.
6. Incubate at 25°C for 10 minutes.
7. Analyze the fragments via gel-electrophoresis or Agilent Bioanalyzer.

## Ordering Information

| Item Number | Size |
|---|---|
| C9-10-0.25 | 0.25mg |
| C9-10-1 | 1mg |

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