



## EnGen® sgRNA Synthesis Kit, *S. pyogenes* (#E3322S)

Engen® sgRNA Synthesis Kit, *S. pyogenes* 는 빠른 시간내에( <1hr) microgram 단위의 custom sgRNA를 합성 할 수 있는 kit로써, single tube reaction format으로 사용하기 간편하게 제공됩니다.

Target-specific한 oligonucleotide(~55nt ssDNA)와 Reaction & Enzyme Mix를 사용하여 간편하게 sgRNA를 합성하며, DNA amplification과 template clean up step이 필요없어 시간을 단축 할 수 있습니다.

Target specific한 DNA oligonucleotide는 NEB의 EnGen® sgRNA Template Oligo Designer (<http://nebiocalculator.neb.com/#!/sgrna>)를 사용하거나 다른 DNA oligonucleotide supplier를 통해 주문하여 사용하시면 됩니다.

sgRNA는 CRISPR/Cas9-based genome editing과 *in vitro* DNA cleavage 실험에 사용됩니다.

### 장점

- Protocol 시간 단축 (Single - reaction format)
- 25 ug sgRNA까지 합성
- Control oligo 제공



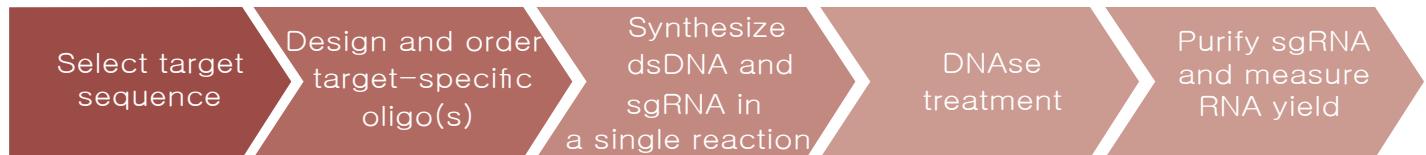
### 주문 정보

| PRODUCT  | NEB #       | SIZE          |
|--|-------------|---------------|
| EnGen® sgRNA Synthesis Kit, <i>S. pyogenes</i>   | E3322S      | 20 rxns       |
| 구성품  | Storage(°C) | Concentration |
| EnGen® sgRNA Enzyme Mix                          | -20         |               |
| EnGen® 2X sgRNA Reaction Mix, <i>S. pyogenes</i> | -20         |               |
| DNase I (RNase-free)                             | -20         | 2 units/ul    |
| EnGen® sgRNA Control Oligo, <i>S. pyogenes</i>   | -20         |               |

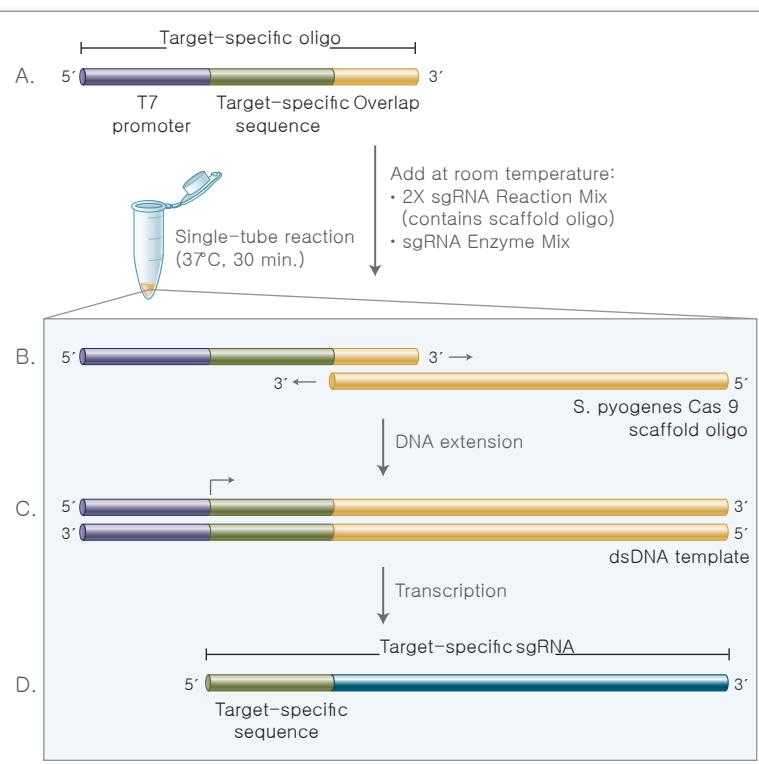
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## EnGen® sgRNA Synthesis Kit workflow



## EnGen® sgRNA Synthesis Kit 개요



A. The Target - specific oligo contains the T7 promoter sequence, ~20 nucleotides of target - specific sequence and a 14 - nucleotide overlap sequence complementary to the *S. pyogene* Cas9 Scaffold Oligo, supplied in the reaction mix. Target-specific oligos are mixed with the EnGen 2X sgRNA reactoin Mix and the EnGen sgRNA Enzyme Mix at room temperature.

B. At 37 ° C the two oligos anneal at the 14- nucleotide overlap region of complementarity.

C. The DNA polymerase contained in the EnGen sgRNA Enzyme Mix extends both oligos from their 3' end, creating a dsDNA template.

D. The RNA polymerase contained in the EnGen sgRNA Enzyme Mix recognizes the dsDNA of the T7 promoter and initiates transcription, resulting in a target-specific sgRNA.

All steps occur in a single reactoin during a 30-minute incubation at 37 ° C.



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