NEXTFLEX™ Cas9-gRNA rRNA Depletion Enzyme (HMR)

Specifications

Catalogue Number	NOVA-801100	NOVA-801500
Units	6 – 100	30 – 500
NEXTFLEX™ Cas9-gRNA Depletion Enzyme volume	50 µL	250 µL
NEXTFLEX™ 10x Cas9 Buffer volume	200 µL	1000 µL
Cas9-guide RNA concentration	5.6 µM	
Storage and stability	12 months from date of manufacture at -80ºC	

Description

NEXTFLEX™ Cas9-gRNA rRNA Depletion Enzyme (HMR) is programmed to deplete rRNA genes specific to mammalian cells (human, mouse, rat). The enzyme shows good depletion performance also with dog and chicken libraries.

NEXTFLEX™ Cas9-gRNA rRNA Depletion Enzyme (HMR) contains 435 unique guide RNAs targeting GRCh38-annotated genes of the nuclear 5S, 5.8S, 18S, 28S and 45S rRNA subunits and the 12S & 16S mitochondrial rRNA sequences. Full information about target genomic locations can be provided upon request.

One unit of NEXTFLEX™ as9-gRNA rRNA Depletion Enzyme (HMR) is defined as the amount required to deplete 1-10 ng of target dsDNA library (≥400 bp). Depletion has also been observed with up to ~100 ng input, although higher inputs may require optimization of enzyme concentration. NEXTFLEX™ Cas9-gRNA rRNA Depletion Enzyme (HMR) should be thawed, diluted with Cas9 buffer if necessary, gently pipette mixed and allowed to sit at room temperature for 10 minutes prior to beginning the reaction.

Recommended Method of Use

Maintaining a ratio of 0.5 – 8.2 µL of NEXTFLEX™ Cas9-gRNA Depletion Enzyme to 1-10 ng dsDNA is recommended. Depletion reaction is prepared as indicated below, where low use case corresponds to 0.5 µL of enzyme (minimum recommended) and high use case corresponds to the addition of 8.2 µL of enzyme. The exact amount of enzyme will depend on the sample that is being studied and needs to be determined by the user.

	Low Use Case	**Mid Use Case**	**High Use Case**
dsDNA (1-10 ng)	17.6 µL*	14.4 µL*	10.8 µL*
Cas9-guide RNA Depletion Enzyme	0.5 µL	4.1 µL	8.2 µL
10x Cas9 Buffer	1.9 µL	1.5 µL	1.0 µL
Total Volume	**20 µL**	**20 µL**	**20 µL**

*Nuclease-free water can be used for volume adjustments

Note: 1 µL of 10X Cas9 Buffer should be added for every 10 µL of total reaction volume above 10 µL (the Cas9-guide RNA complex already contains 1 µL of 10X Cas9 Buffer). Please note: a reaction volume >30 µL is not recommended. The Cas9-gRNA Depletion Enzyme contains RNase inhibitor, so additional RNase inhibitor need not be included in the reaction.

Incubate the above reaction for 1 hour at 42°C, then transfer to ice for ~ 2 minutes. Cas9-gRNA Depletion Enzyme treatment should be followed by SPRI bead-based size selection (0.6-0.8X if using AMPure XP or NEXTFLEX™ NGS Cleanup beads) to remove cleaved material and PCR to amplify intact library fragments, usually also followed by 0.6-0.8X if using AMPure XP or NEXTFLEX™ NGS Cleanup beads to remove smaller products before sequencing. The number of PCR cycles will depend on the quality and quantity of input dsDNA products

Quality Control

Functionality of the Cas9-guide RNA complex is determined by testing depletion of an NGS library using total human RNA as input. Depleted libraries are sequenced on an Illumina instrument, and the data is analyzed to determine the % of rRNA reads as compared to the total number reads.