

## 2019nCoV (SARS-CoV-2) Nsp10/Nsp16 complex

Nsp10: nsp10-CysHis, GFL protein

Nsp16: 2'-O-ribose methyltransferase, 2'-o-MT

### About Coronavirus Nsp16

Nsp16 is an SAM-dependent nucleoside-2'O-methyl-transferase (2'O-MTase). The mRNA cap for coronaviruses is completed by Nsp16, which ensures formation of a protective cap-1 structure that prevent recognition by either MDA5 or IFIT proteins. Finally, the NSP16/NSP10 complex finishes coronavirus capping process permitting viral infection with reduced host recognition.<sup>1</sup>

### About Coronavirus Nsp10

Nsp10 (18 kDa) is well conserved among coronaviruses and encoded by ORF1a. It's thought to serve as an important multifunctional cofactor in replication. Nsp10 was shown to interact with itself, as well as with Nsp1, Nsp7, Nsp14, and Nsp16. The important role of Nsp10 is responsible for RNA synthesis. It was shown that a murine hepatitis virus (MHV) temperature-sensitive mutant carrying a non-synonymous mutation in the Nsp10 coding sequence had a defect in minus-strand RNA synthesis at non-permissive temperatures<sup>1</sup>.

### About Coronavirus Nsp10/Nsp16 complex

When bound to nsp10, nsp16 is active as a type-0 RNA cap-dependent 2'O-MTase, ie., active only when the cap guanine is methylated at its N7 position. The nsp10/nsp16 crystal structure shows that nsp16 adopts a typical fold of the S-adenosylmethionine-dependent methyltransferase family as defined initially for the catechol OMTase.<sup>1,2</sup>

### High-throughput screening (HTS) assay development of Coronavirus Nsp10/Nsp16 complex 2'O-MTase for antiviral compounds

HTS Approach: Radioactive Methyltransferase Assay

Simple 2019nCoV (SARS-CoV-2) Nsp10/Nsp16 complex 2'O-MTase activity assay protocol

2019nCoV (SARS-CoV-2) Nsp10/Nsp16 complex MTase activity assays were performed in 40 mM Tris-HCl, pH 8.0, 5 mM DTT, 1 mM MgCl<sub>2</sub>, 2 mM 7MeGpppAC5 or GpppAC5, 10 mM AdoMet, and 0.03 mCi/ml [3H] AdoMet .






Short capped RNAs (7MeGpppAC5, GpppAC5, were synthesized in vitro using bacteriophage T7 DNA primase and were purified by high-performance liquid chromatography (HPLC). In the standard assay, nsp10 and nsp16 were added at final concentrations of 600 nM, and 200 nM, respectively, and the amount of 3H-CH<sub>3</sub> transferred onto 7MeGpppAC5 substrates was determined by filter binding assay<sup>2</sup>.

## References

- 1 Gordon, D. E. *et al.* A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. *BioRxiv*, doi:10.1101/2020.03.22.002386 (2020).
- 2 Decroly, E. *et al.* Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-O-methyltransferase nsp10/nsp16 complex. *PLoS Pathog* 7, e1002059, doi:10.1371/journal.ppat.1002059 (2011).

## SARS-CoV-2 (2019nCoV) Non-structure protein (Nsp) Recombinant Antigens

- Recombinant Proteins Of SARS-CoV-2 (2019nCoV) Drugable Target For High-throughput screening (HTS) assay development of antiviral compounds against COVID-19

Name of Non-structure protein of SARS-CoV-2 (2019-nCoV, novel coronavirus)	Name of Gene in Coronavirus	GeneMedi's Recombinant Antigens For Activity Assay	High-throughput screening (HTS) assay development information and protocols
(PLpro) papain-like proteinase	Nsp3	<a href="#">GMP-V-2019nCoV-PLpro001</a>	Download 
Mpro (main protease,3CLpro)	Nsp5	<a href="#">GMP-V-2019nCoV-Mpro001</a>	Download 
Nsp10-CysHis,GFL protein	Nsp10	<a href="#">GMP-V-2019nCoV-Nsp10-01</a>	Download 
RNA-dependent RNA polymerase(RdRP)	Nsp12	<a href="#">GMP-V-2019nCoV-RdRP001</a>	Download 
2'-O-ribose methyltransferase	Nsp16	<a href="#">GMP-V-2019nCoV-Nsp16-01</a>	Download 
Nsp3-X domain(Macro domain)	Nsp3	<a href="#">GMP-V-2019nCoV-Nsp3X-01</a>	