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Screening compounds that inhibit SARS-CoV-2 nsp3 macrodomain: ADP-Ribose quantification by LC/MS-MS

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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Abstract

Functional biochemical assay to identify treatments for viral infectious disease that targets SARS-CoV-2 nsp3 macrodomain (MAC1). Utilizing Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis, MAC1 ability to hydrolyze αNAD+ in the presence of potential inhibitors was measured by quantifying ADPR and αNAD+ levels. This assay is characterized by the relative potency of inhibitors as IC50, it is modified from https://doi.org/10.1126/sciadv.abf8711. In this version, we have added the Addgene id.

Materials

Construct used

Addgene: Sars Cov 2 Mac domain Sars Cov 2 Mac domain Plasmid **addgene Catalog #**204787

Assay Reagents (Concentration listed are from Stock Solutions)

1. [M] 250 millimolar (mM) HEPES 0.5M buffer soln. pH 7.0 **Fisher Scientific Catalog #**AAJ60064AE (or similar)
2. [M] 200 millimolar (mM) Sodium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9888
3. [M] 0.5 mg/mL Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7030
4. α-Nicotinamide adenine dinucleotide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N6754
5. Adenosine 5′-diphosphoribose sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A0752

Protocol materials

α-Nicotinamide adenine dinucleotide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N6754

Sodium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9888

Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7030

Adenosine 5′-diphosphoribose sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A0752

Sars Cov 2 Mac domain Plasmid **addgene Catalog #**204787

HEPES 0.5M buffer soln. pH 7.0 **Fisher Scientific Catalog #**AAJ60064AE

Safety warnings

Please be sure to wear proper Personal Protective Equipment (PPE) while performing this experiment.

Before start

Note: Inhibitor compounds stock concentration is 20 mM. Compounds are pre-dispensed into assay plates and stored at -20˚C until use.

SARS-CoV-2 nsp3 expression and purification

1 **The protein used in this assay was expressed and purified according to this protocol:**
We used the following plasmid in the protein expression and purification protocol.
Sars Cov 2 Mac domain Plasmid **addgene Catalog** #204787

Protocol

NAME

SARS-CoV-2 nsp3 macrodomain His-tagged expression and purification protocol for assay

CREATED BY

korvus.wang

Preview

Assay reagents preparation

2 **Assay buffer:**

	A	B	D	E
		Stock	**Final in assay plate**	**Units**
	HEPES pH=7.0	1000	25	mM
	NaCl	5000	100	mM
	BSA	10	1	mg/ml

	A	B	C	D	E
	Reagent	**Stock**	**dispensed to plate**	**Final in assay plate**	**Units**
	His-SARS-CoV-2 MAC1	183000	800	200	nM

	A	B	C	D	E
	Substrate (NAD+)	Dry (disslove fresh for each assay)	32	8	µM

Prepare 96 well-plate

1m

3 **PRIME** the dispenser with **Assay Buffer** by selecting the **PRIME** button until the tubes are filled completely.

3.1 **DISPENSE** 75 µL Mac1 assay Buffer to control wells of assay plate and 50 µL Mac1 Buffer to the rest of the plate.

- **Note:** Control wells are for **NAD$^+$ alone** and **MAC1+NAD$^+$**, without compounds

3.2 **EMPTY** the dispenser tubes.

- Discard the liquid discharged from the tubes.

4 **PRIME** the dispenser with **4x MAC1 enzyme** (800 nM) by selecting the **PRIME** button until the tubes are filled completely.

4.1 **DISPENSE** 25 µL 4xMac1 to all wells of assay plate besides **NAD$^+$ alone** control well.

4.2 **EMPTY** the dispenser tubes.

- Discard the liquid discharged from the tubes.

5 **CENTRIFUGE** plate 1500 rpm, Room temperature, 00:01:00 to remove bubbles 1m

6 **INCUBATE** plate for 00:15:00 at Room temperature 15m

7 **PRIME** the dispenser with **4x NAD$^+$** (32 µM) by selecting the **PRIME** button until the tubes are filled completely.

7.1 **DISPENSE** 25 µL 4xNAD+ to all wells of assay plate.

7.2 **EMPTY** the dispenser tubes.
- Discard the liquid discharged from the tubes.

8 **CENTRIFUGE** plate 1500 rpm, Room temperature, 00:01:00 to remove bubbles 1m

9 **INCUBATE** plate on an Orbi-plate shaker at 300 rpm for 02:45:00 at Room temperature 2h 45m

10 **STOP** the reaction by freezing the plate and store in -80 until LC/MS analysis.

LC/MS-MS analysis

11 Quantitative analysis of ADPR and αNAD+ was performed as was recently published at Lee, Alpha A et al. "Discovery of potent SARS-CoV-2 nsp3 macrodomain inhibitors uncovers lack of translation to cellular antiviral response." *bioRxiv : the preprint server for biology* 2024.08.19.608619. 21 Aug. 2024, doi:10.1101/2024.08.19.608619. Preprint. **https://pmc.ncbi.nlm.nih.gov/articles/PMC11370477/**

Protocol references

https://doi.org/10.1126/sciadv.abf8711