SARS-CoV-2 nsp3 Mac1 macrodomain TR-FRET Peptide displacement Assay V.2

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ASAP Discovery

VERSION 2

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

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DISCLAIMER

ABSTRACT

This is a HTRF-based peptide displacement assay

Experiment Concentrations (From Stock to Assay)

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	His-SARS COV2 MAC1	183000	50	12.5	nM
	Substrate (Biotin-ADPr)	10000000	1600	400	nM
	Detection solution				
	Streptavidin-XL665 (SA-XL)	1	0.25	0.125	%

	A	B	C	D	E
	MAb Anti-6HIS-Eu cryptate Gold	100	0.25	0.125	%
	Assay buffer				
	HEPES pH=7.0	250	25	25	mM
	NaCl	200	20	20	mM
	BSA	0.5	0.05	0.05	%
	Tween 20	0.5	0.05	0.05	%
	HTRF PPI Europium Detection Buffer	100	10	10	%

For more information, please check out the "Materials" Section

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Compound Plate Design for Dose Response:

Total Assay Volume: 16 µL
Compounds Top Assay Concentration: 100 µM
Dilution Factor: 3
Dose Response Points: 10
Number of Replicates: 2
Backfill with DMSO: Yes

Compounds Plate Design for 2-Point Assay:

Total Assay Volume: 16 µL
Compounds Assay Concentration: 100 µM and 50µM
Dilution Factor: 2
Dose Response Points: 2
Number of Replicates: 2
Backfill with DMSO: Yes

MATERIALS

Assay Buffer 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**
   (or similar)

3. [M] 0.5 % volume
   Bovine Serum Albumin (BSA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7030**
4. [M] 0.5 % volume
   TWEEN® 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416**
5. [M] 100 % volume
   HTRF PPI Europium Detection Buffer **CISBIO BIOASSAYS (PerkinElmer) Catalog #61DB9RDF**

***Note:** *There are several forms of the Assay Buffer in this experiment.* The **Assay Buffer** is the final, active buffer used throughout the experiment and has all of the five above reagents included. **HTRF PPI Europium Detection Buffer** needs to be added fresh before each experiment. Thus, there was an intermediate Buffer called **Mac1 Buffer** that contained HEPES, NaCl, BSA, and Tween only. Mac1 Buffer was filtered and stored at 4˚C. **HTRF PPI buffer** was then added to Mac1 Buffer fresh (to a final concentration of 10%) prior to performing the experiment--creating **the active Assay Buffer.**

Detection Solution Reagents (Concentration listed are from Stock Solutions)

[M] 1 % volume
Streptavidin-XL665 **CISBIO BIOASSAYS (PerkinElmer) Catalog #610SAXAC**

- **Note:** Streptavidin-XL665 was dissolved in triply distilled water and diluted with HTRF PPI buffer to its stock concentration and then was aliquoted into 1.5mL sterile conical tubes

[M] 100 Mass Percent
MAb Anti-6HIS-Eu cryptate Gold **CISBIO BIOASSAYS (PerkinElmer) Catalog #61HI2KLA**

Note: MAb Anti-6HIS-Eu cryptate Gold was dissolved in tripled distilled water and then aliquoted into 1.5mL sterile conical tubes

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Additional Reagents:

[M] 183000 nanomolar (nM) **His-SARS COV2 MAC1 Enzyme**

- The Enzyme original stock was originally [M] 183000 nanomolar (nM) and was diluted to [M] 50 nanomolar (nM) before every experiment in **freshly made Assay Buffer**. The final assay concentration is [M] 12.5 nanomolar (nM)

[M] 10000000 nanomolar (nM) **Substrate (Biotin-ADPr) MAC1**

- Substrate stock (ARTK(Bio)QTARK(Aoa-RADP)S) was dissolved in DMSO to the stock concentration. Before each experiment, the Substrate STock was diluted to [M] 1600 nanomolar (nM) in freshly made Assay Buffer.

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

Note: Inhibitor compounds stock concentration is [M] 20 millimolar (mM) . Compounds are pre-dispensed into 384 plates and stored at -20˚C until use.

Prepare Reagents

1 **PREPARE** all of the reagents/buffers required for this experiment.

Reagents

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	His-SARS-CoV-2 MAC1	183000	50	12.5	nM
	Substrate (Biotin-ADPr)	10000000	1600	400	nM

Detection Solution

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	Streptavidin-XL665 (SA-XL)	1	0.25	0.125	%
	MAb Anti-6HIS-Eu cryptate Gold	100	0.25	0.125	%

MAC1 Buffer

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	HEPES pH=7.0	250	25	25	mM
	NaCl	200	20	20	mM
	BSA	0.5	0.05	0.05	%
	Tween 20	0.5	0.05	0.05	%

HTRF PPI Europium Detection Buffer

	A	B	C	D	E

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	HTRF PPI Europium Detection Buffer	100	10	10	%

Assay Buffer

	A	B	C	D	E
	Reagent	**Stock**	**Loaded into Combi**	**Final in assay plate**	**Units**
	HEPES pH=7.0	250	25	25	mM
	NaCl	200	20	20	mM
	BSA	0.5	0.05	0.05	%
	Tween 20	0.5	0.05	0.05	%
	HTRF PPI Europium Detection Buffer	100	10	10	%

Prepare 384-well Plate

16m

2 **PRIME** Multi-Drop Combi Tube Dispensing Cassette **MAC1 Buffer** by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).

2.1 **DISPENSE** 4 µL Mac1 Buffer to Columns **1 and 23** of assay plate

- **Note:** These will represent the ***inhibitor control columns***

2.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard** the MAC1 Buffer discharged from the cassette.

3 **PRIME** Multi-Drop Combi Tube Dispensing Cassette **His-SARS COV2 MAC1 Enzyme** by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).

3.1 **DISPENSE** 4 µL [M] 50 nanomolar (nM) His-SARS COV2 MAC1 Enzyme to Columns **1 and 23** of assay plate

Note:

- [M] 50 nanomolar (nM) His-SARS COV2 MAC1 is four times the final concentration for the assay. It will be diluted to be a final concentration of [M] 12.5 nanomolar (nM) His-SARS COV2 MAC1 Enzyme
- Column **2** and Column **24** are ***neutral control columns*** (Contain: Enzyme, Substrate, DMSO; **no experimental compounds**)

3.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard** the [M] 50 nanomolar (nM) His-SARS COV2 MAC1 Enzyme discharged from the cassette.

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

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

6 **PRIME** Multi-Drop Combi Tube Dispensing Cassette [M] 1600 nanomolar (nM) MAC1 Substrate (Biotin-ADPr) by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).

6.1 **DISPENSE** 4 µL [M] 1600 nanomolar (nM) MAC1 Substrate (Biotin-ADPr) into Columns **1 through 23 and 24** (the full plate)

Note:

- [M] 1600 nanomolar (nM) MAC1 Substrate (Biotin-ADPr) is four times the final concentration for the assay. It will be diluted to be a final concentration of [M] 400 nanomolar (nM) MAC1 Substrate (Biotin-ADPr)

6.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard** the [M] 1600 nanomolar (nM) MAC1 Substrate (Biotin-ADPr) discharged from the cassette.

7 **CENTRIFUGE** 1500 rpm, Room temperature, 00:01:00 plate to remove bubbles

8 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with **Assay Buffer** by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely. Then, **EMPTY** the Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard the Assay Buffer discharged from the cassette.**

9 **PRIME** Multi-Drop Combi Tube Dispensing Cassette [M] 0.25 % volume Detection Solution by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).

9.1 **DISPENSE** 8 µL [M] 0.25 % volume Detection Solution into full plate

Note:

- [M] 0.25 % volume Detection Solution is two times the final concentration for the assay.

  It will be diluted to be a final concentration of [M] 0.125 % volume Detection Solution

9.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard** the [M] 1600 nanomolar (nM) MAC1 Substrate (Biotin-ADPr) discharged from the cassette.

10 **CENTRIFUGE** 1500 rpm, Room temperature, 00:01:00 plate to remove bubbles

11 **INCUBATE** plate for 01:00:00 at Room temperature 1h

Recommended: Clean/Empty the Multi-Drop Combi Reagent Dispenser and Dispensing Cassette during this incubation step

Reat Plate Fluorescence

12 **READ** and **RECORD** the plate Relative fluorescence units (RFU) via the "**Mac1 Protocol**" on the PHERAstar FS Control Software.

Equipment

PHERAstar FS	NAME
Microplate reader	TYPE
BMG LABTECH	BRAND
0471B0001A	SKU
https://www.bmglabtech.com/en/pherastar-fsx/?utm_term=pherastar%20plate%20reader&utm_campaign=usa.roi.products&utm_source=adwords&utm_medium&gclid=Cj0KCQjw8qmhBhClARIsANAtbodGRjigZtEYwcoMXUtxsLn25xp4gjKra3ZNt9jLh9-FwOoFR_5EUHUaAlkREALw_wcB	LINK

Expected result

Donor 325/620 ex/em should be ~ 5000 . Acceptor ~ 3000