APR 19, 2023

OPEN ACCESS

Protocol Citation: Haim Barr, Noa Lahav 2023. SARS-CoV-2 Macrodomain (Mac1) TR-FRET Dose Response. **protocols.io** https://protocols.io/view/sars-cov-2-macrodomain-mac1-tr-fret-dose-response-cssfwebn

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

Created: Apr 12, 2023

Last Modified: Apr 19, 2023

PROTOCOL integer ID: 80423

SARS-CoV-2 Macrodomain (Mac1) TR-FRET Dose Response

Haim Barr[1,2], Noa Lahav[1,2]

[1]The Weizmann Institute of Science; [2]ASAP Discovery

ASAP Discovery

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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	%
	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

GUIDELINES

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 Top Assay Concentration: 100 µM
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 #610SAXA(**

- **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

---

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**

	A	B	C	D	E
	His-SARS COV2 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
	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