



## HTRF Thalidomide - Red ligand

Part # 64BDCRBNRED

**Test Size#:** 10 binding assays (K<sub>d</sub> determination) - assay volume: 20 µL

**Revision:** #03 of October 2025      **Store at:** ≤-16°C

**This product is intended for research purposes only. It is not intended to be used for therapeutic or diagnostic purposes.**

### ASSAY PRINCIPLE

Revvity<sup>®</sup> HTRF Thalidomide-Red ligand (#Revvity 64BDCRBNRED) is primarily intended to perform affinity binding curves using HTRF<sup>®</sup> technology and can be used in association with the HTRF Cereblon binding kit (#Revvity 64BDCRBNPEG/H).

The HTRF Thalidomide-Red ligand is particularly suited for cooperativity binding studies to assess the effect of a PROTAC protein substrate on the affinity of the Thalidomide-Red ligand for Cereblon protein. HTRF Thalidomide-Red ligand binding is detected in a direct binding assay format using an anti GST antibody labeled with Europium Cryptate which binds to Human Cereblon WT GST-tagged. When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The specific binding signal is calculated by subtracting the non-specific binding signal from the total signal, enabling determination of K<sub>d</sub> for HTRF Thalidomide-Red ligand (Fig. 1).

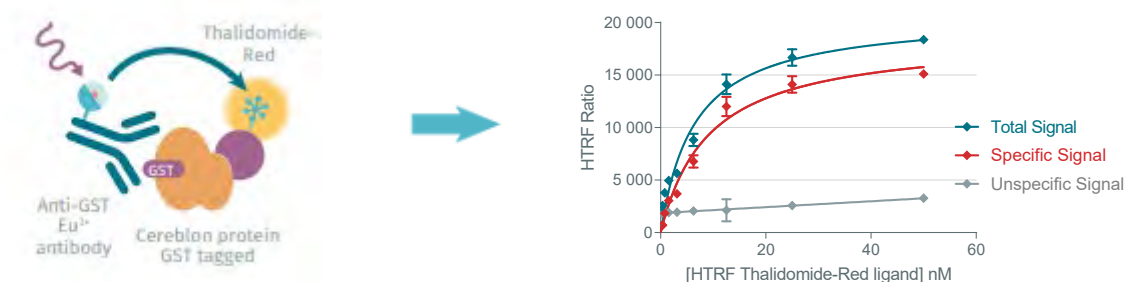
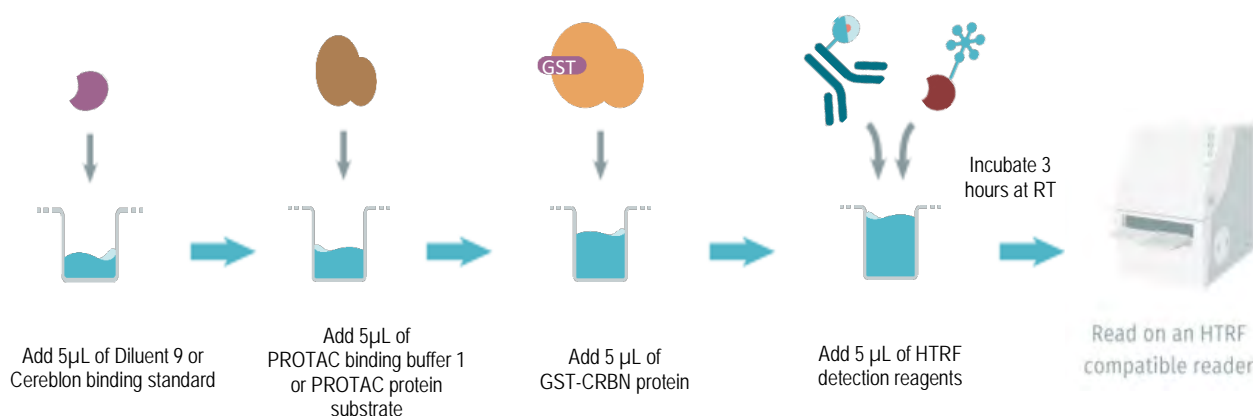


Figure 1: Principle of K<sub>d</sub> determination with HTRF Thalidomide-Red ligand.

### MANUAL AT A GLANCE



Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

Make sure you use the appropriate setup for Eu 3+ Cryptate. For more information about setup and HTRF<sup>®</sup> compatible readers, please visit our website at: [www.revvity.com](http://www.revvity.com)

**MATERIAL PROVIDED:**

HTRF Thalidomide - Red ligand - 5X	1 vial - 200 µL Frozen	
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**PURCHASE SEPARATELY:**

- Cereblon Standard (#Revvity 64BDCRBNCDA)
- mAb anti-GST-Eu cryptate Kinase Binding (#Revvity 62KBGSTKAF/B)
- HTRF PROTAC binding buffer 1 (#Revvity 64BDE31RDF)
- Diluent 9 (5X) (#Revvity 62DL9DDA/C)
- HTRF Cereblon binding kit (#Revvity 64BDCRBNPEG/H)
- Low volume white (only) microplate\*
- HTRF®-Certified Reader \*\*. Make sure the setup for Eu3+ Cryptate is used.

\* For HTRF microplate recommendations, please visit [www.revvity.com](http://www.revvity.com)

\*\* For a list of HTRF-compatible readers and setup recommendations, please visit [www.revvity.com](http://www.revvity.com)

**STORAGE AND STABILITY**

Store the HTRF Thalidomide - Red ligand at -16°C or below. Under appropriate storage conditions, HTRF Thalidomide - Red ligand is stable until the expiry date indicated on the label.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.

Volume of HTRF Thalidomide - Red ligand aliquots should not be under 10 µL.

Once thawed, aliquoted HTRF Thalidomide - Red ligand can be frozen once.

**REAGENT PREPARATION****BEFORE YOU BEGIN:**

- It is very important to prepare all reagents in the specified buffers. The use of incorrect buffers may affect reagent stability and assay results.
- Cereblon binding standard will be used to determine the non-specific binding signal.
- PROTAC binding buffer 1 is ready-to-use.
- GST-CRBN protein must be prepared following instructions from the package insert of the HTRF CRBN binding kit.
- Diluent 9 (5X) must be homogenized with a vortex then diluted 5-fold with distilled water (e.g. 200µL of Diluent 9 (5X) + 800µL of distilled water) and mixed gently to obtain the Diluent 9 (1X).

► **CEREBLON BINDING STANDARD**

**TO PREPARE THE CEREBLON BINDING STANDARD STOCK SOLUTION:**

Thaw the Cereblon binding Standard. Centrifuge.

This 8mM stock solution can be frozen and stored at -16°C or below.

**TO PREPARE THE CEREBLON BINDING STANDARD WORKING SOLUTION:**

Homogenize the Cereblon binding Standard stock solution then dilute it 20-fold with Diluent 9 (1X) (e.g. 10µL of Cereblon binding Standard stock solution + 190µL Diluent 9 (1X)). Mix gently.

## ► HTRF THALIDOMIDE-RED LIGAND

### TO PREPARE THE HTRF THALIDOMIDE-RED LIGAND STOCK SOLUTION:

Thaw the HTRF Thalidomide-Red ligand. Centrifuge.

This 5X stock solution can be frozen and stored at -16°C or below.

### TO PREPARE THE HTRF THALIDOMIDE-RED LIGAND WORKING SOLUTION:

The HTRF Thalidomide-Red ligand working solution (C7) is obtained by diluting 5-fold the 5X stock solution of HTRF Thalidomide-Red ligand with PROTAC binding buffer 1 e.g. 20µL of thawed HTRF Thalidomide-Red ligand stock solution + 80µL of PROTAC binding buffer 1.

Use the HTRF Thalidomide-Red ligand working solution to prepare the recommended range of concentrations by using serial dilutions (in order to counteract any sticking, we recommend changing tips between each dilution).

Procedure for preparing 1 range of HTRF Thalidomide-Red ligand (for monitoring total and non-specific binding signals):

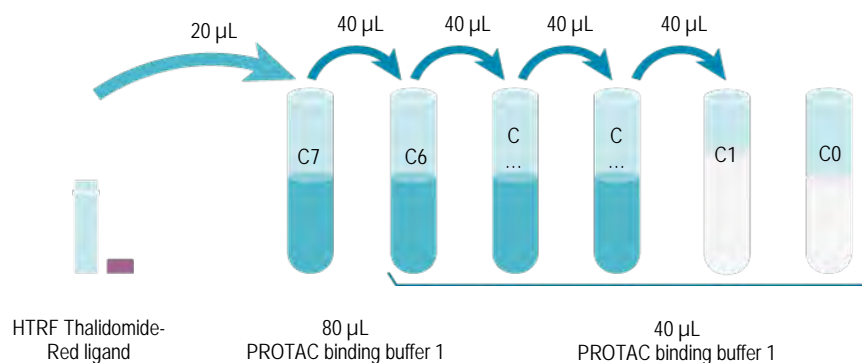
Prepare 100µL of HTRF Thalidomide-Red ligand working solution (C7).

Use the high concentration (C7) to prepare the range of concentrations using ½ serial dilutions as follows:

- Dispense 40µL of PROTAC binding buffer 1 into each vial from C6 to C0.

Add 40µL of C7 of HTRF Thalidomide-Red ligand to 40µL of PROTAC binding buffer 1 to obtain the C6, mix gently and repeat the ½ serial dilution to make the concentrations: C5, C4, C3, C2 and C1. C0 (Negative control) is PROTAC binding buffer 1 alone.

This will create 7 concentrations of HTRF Thalidomide-Red ligand to be used for Total and non-specific binding conditions.



HTRF THALIDOMIDE-RED	SERIAL DILUTIONS	"HTRF THALIDOMIDE-RED WORKING SOLUTION (nM)"	" HTRF THALIDOMIDE-RED FINAL CONCENTRATION (nM)"
Standard Stock solution	Thawed stock solution	2 000	-
C7	20µl stock solution + 80 µL PROTAC binding buffer 1	400	50
C6	40 µL C7 + 40 µL PROTAC binding buffer 1	200	25
C5	40 µL C6 + 40 µL PROTAC binding buffer 1	100	12.5
C4	40 µL C5 + 40 µL PROTAC binding buffer 1	50	6.25
C3	40 µL C4 + 40 µL PROTAC binding buffer 1	25	3.125
C2	40 µL C3 + 40 µL PROTAC binding buffer 1	12.5	1.562
C1	40 µL C2 + 40 µL PROTAC binding buffer 1	6.25	0.781
C0	80 µL PROTAC binding buffer 1	0	0

► **MAB ANTI-GST EU CRYPTATE KINASE BINDING:**

**TO PREPARE THE MAB ANTI-GST EU CRYPTATE KINASE BINDING STOCK SOLUTION:**

Thaw the mAb anti-GST Eu cryptate Kinase Binding. Centrifuge.

This stock solution can be frozen and stored at -16°C or below.

**TO PREPARE THE MAB ANTI-GST EU CRYPTATE KINASE BINDING WORKING SOLUTION:**







Homogenize the mAb anti-GST Eu cryptate Kinase Binding stock solution then dilute it 50-fold with PROTAC binding buffer 1 (e.g. 40µL of anti-GST Eu cryptate stock solution + 1960µL of PROTAC binding buffer 1). Mix gently.

**WARNING:** Do not prepare the mAb anti-GST Eu cryptate Kinase Binding working solution following instructions from the package insert of the mAb anti-GST-Eu cryptate Kinase Binding (#Revvity 62KBGSTKAF/B).

► **PRE-MIXED SOLUTION OF HTRF THALIDOMIDE-RED LIGAND + MAB ANTI-GST EU CRYPTATE KINASE BINDING:**

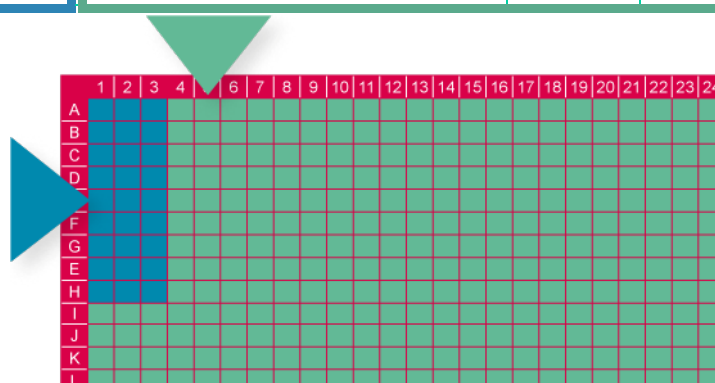
Just prior dispensing, pre-mix the working solutions of HTRF Thalidomide-Red ligand and mAb anti-GST Eu cryptate Kinase Binding by adding 1 volume of GST Eu cryptate antibody Kinase Binding working solution to 1 volume of each HTRF Thalidomide-Red ligand working solutions (C7 to C0) (e.g. 25µL of HTRF Thalidomide-Red ligand working solution + 25µL of GST Eu cryptate antibody Kinase Binding working solution).

**ASSAY MANUAL**

	NON-SPECIFIC BINDING SIGNAL	TOTAL BINDING SIGNAL
<b>Step 1</b> 	Dispense 5 µL of Cereblon binding Standard into each well	Dispense 5 µL of PROTAC binding buffer 1 into each well
<b>Step 2</b> 	Add 5 µL of PROTAC binding buffer 1 or 5µL of PROTAC substrate protein (diluted in the PROTAC binding buffer 1)	
<b>Step 3</b> 	Add 5µL of GST-CRBN protein	
<b>Step 4</b> 	Add 5 µL of premixed HTRF Thalidomide-Red ligand and mAb GST Eu Kinase Binding working solutions to all wells	
<b>Step 5</b> 	Seal the plate and incubate 3 hours at RT	
<b>Step 6</b> 	Remove the plate sealer and read on an HTRF® compatible reader	

## EXAMPLE OF MAP PLATE

	NON-SPECIFIC BINDING			TOTAL BINDING		
	1	2	3	4	5	6
A	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C0 and mAb GST Eu Kinase Binding premixed	Repeat Well A1	Repeat Well A1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C0 and mAb GST Eu Kinase Binding premixed	Repeat Well A4	Repeat Well A4
B	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C1 and mAb GST Eu Kinase Binding premixed	Repeat Well B1	Repeat Well B1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C1 and mAb GST Eu Kinase Binding premixed	Repeat Well B4	Repeat Well B4
C	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C2 and mAb GST Eu Kinase Binding premixed	Repeat Well C1	Repeat Well C1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C2 and mAb GST Eu Kinase Binding premixed	Repeat Well C4	Repeat Well C4
D	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C3 and mAb GST Eu Kinase Binding premixed	Repeat Well D1	Repeat Well D1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C3 and mAb GST Eu Kinase Binding premixed	Repeat Well D4	Repeat Well D4
E	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C4 and mAb GST Eu Kinase Binding premixed	Repeat Well E1	Repeat Well E1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C4 and mAb GST Eu Kinase Binding premixed	Repeat Well E4	Repeat Well E4
F	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C5 and mAb GST Eu Kinase Binding premixed	Repeat Well F1	Repeat Well F1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C5 and mAb GST Eu Kinase Binding premixed	Repeat Well F4	Repeat Well F4
G	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C6 and mAb GST Eu Kinase Binding premixed	Repeat Well G1	Repeat Well G1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C6 and mAb GST Eu Kinase Binding premixed	Repeat Well G4	Repeat Well G4
H	5 $\mu$ L of Cereblon Binding Standard 5 $\mu$ L of PROTAC binding buffer 1 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C7 and mAb GST Eu Kinase Binding premixed	Repeat Well H1	Repeat Well H1	5 $\mu$ L of diluent #9 5 $\mu$ L of PROTAC binding buffer 1 or PROTAC protein substrate 5 $\mu$ L GST-CRBN protein 5 $\mu$ L of HTRF Thalidomide-Red ligand C7 and mAb GST Eu Kinase Binding premixed	Repeat Well H4	Repeat Well H4



## DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the specific binding signal for each concentration used of HTRF Thalidomide-Red ligand.

$$\text{Specific binding signal} = \text{Total binding signal} - \text{Non-specific binding signal}$$

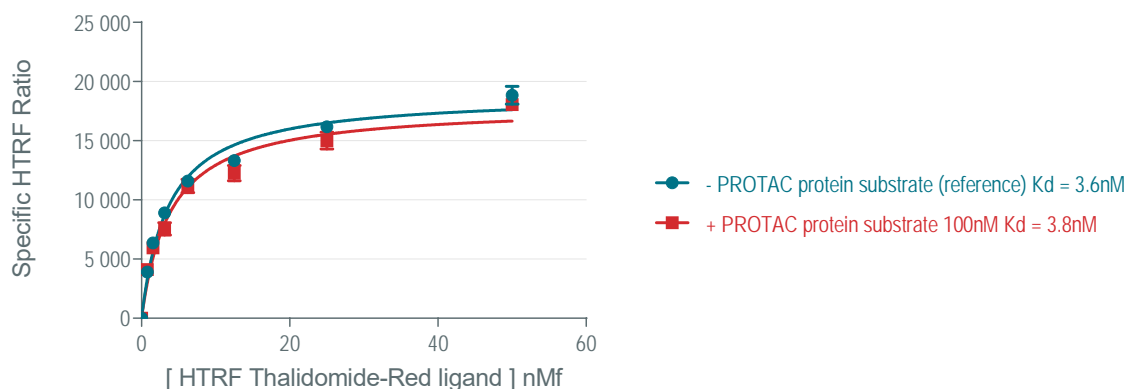
For more information about data reduction, please visit [www.revity.com](http://www.revity.com)

## RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example (readouts on an HTRF compatible reader). Results may vary from one HTRF® compatible reader to another.

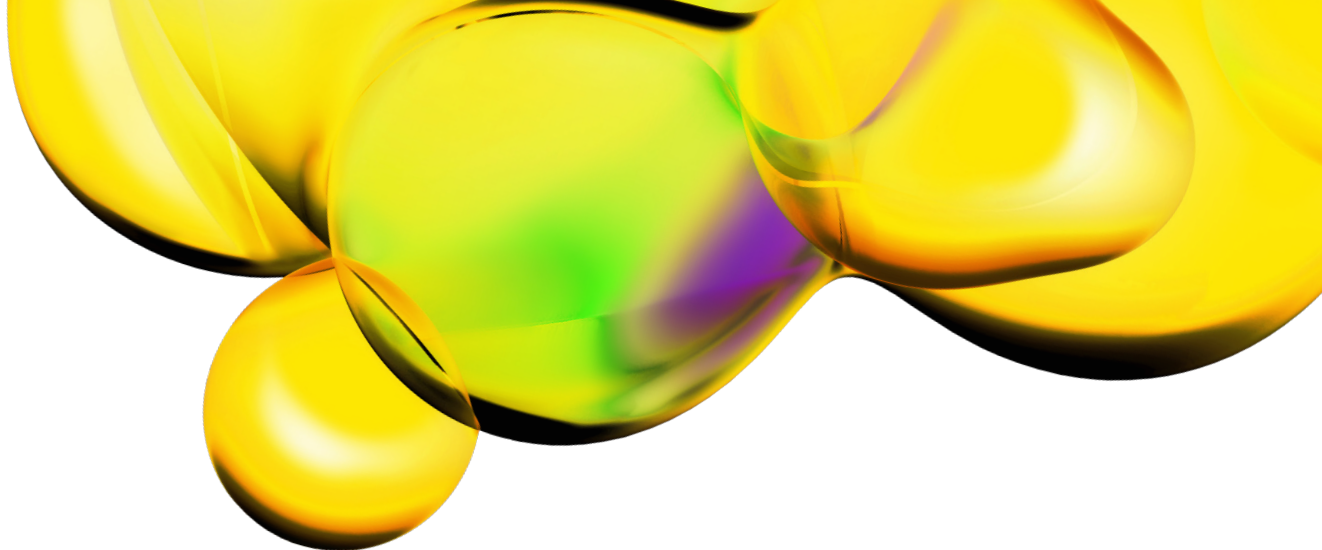
Example of cooperativity binding studies with HTRF Thalidomide-Red ligand to monitor the effect of a PROTAC protein substrate as model at 100nM on the K<sub>d</sub> of HTRF Thalidomide-Red ligand for GST-CRBN protein in the same conditions as the PROTAC compound screening assays performed with the HTRF CRBN binding kit. Results from this example show no effect of this protein substrate.

### Specific binding of HTRF Thalidomide-Red ligand



## ANALYTICAL CHARACTERISTICS

HTRF Thalidomide-Red ligand K <sub>d</sub> (reference without PROTAC protein substrate)	3.2nM ±0.7 (2SD)
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