DUB-Detector™
Deubiquitination Assay

Catalog No. 40110

(version A1)

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TABLE OF CONTENTS **Page**

Overview 1

Flow Chart of Process 2

Introduction 3

Kit Performance and Benefits 5
DUB-Detector Assay 5

Kit Components and Storage 7
Additional Materials Required. 7

Protocols
Buffer Preparation and Recommendations 8
Quick Chart for Preparing Buffers 9
DUB-Detector Protocol 10

References. 13

Appendix
Section A. Troubleshooting Guide. 13
Section B. Related Products 14

Technical Services 16

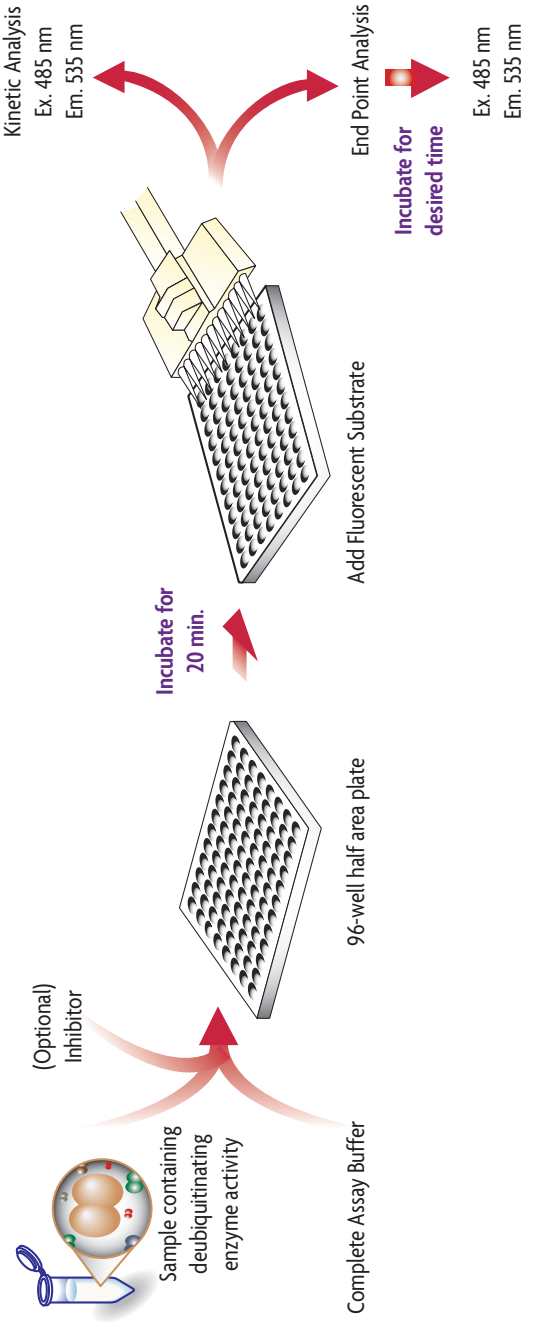
Overview

The DUB-Detector™ Deubiquitination Assay provides a simple solution for screening activity of deubiquitination enzymes from cell extracts or purified recombinant proteins in human, mouse and rat systems. The assay uses a fluorescent ubiquitin substrate that is recognized by the cysteine protease class of deubiquitinating enzymes and hydrolyzed to release a fluorescent signal. The fluorescent signal generated is proportional to the amount of enzymatic activity. DUB-Detector can be used to capture kinetic information about deubiquitinating enzymes, or to screen for potential inhibitor compounds.

The DUB-Detector Kit contains a fluorescent ubiquitin substrate, optimized buffers to enhance enzymatic activity and a 96-well half area plate to perform the reactions. For added convenience, HeLa nuclear extract and an inhibitor of all classes of deubiquitinating enzymes are included as positive and negative controls. The fluorescent signal released by the hydrolysis of the substrate can be detected using a fluorescent microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

product	format	catalog no.
DUB-Detector™	1 x 96 rxns	40110

Flow Chart of Process



Introduction

Deubiquitination Enzymes

Ubiquitination is a reversible, post-translational modification in which the 76 amino acid polypeptide ubiquitin is added to proteins by the sequential action of three enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase. Monoubiquitination is the addition of a single ubiquitin moiety to a protein substrate which is implicated in protein trafficking and DNA repair[1]. Additional ubiquitin molecules can be added to generate a polyubiquitin chain. Proteins that are tagged by ubiquitination are often targeted for ATP-dependent proteolysis by 26S proteasome, but the addition of ubiquitin is also important for regulating protein function, particularly that of histones, and the process has key roles in many cellular signaling cascades[2].

Protein ubiquitination is a highly dynamic process and the removal of ubiquitin from proteins is now considered to be equally important for protein regulation. The large group of enzymes that are responsible for the removal of ubiquitin from proteins are known as deubiquitination enzymes (also known as DUBs, deubiquitylating enzymes or ubiquitin deconjugating enzymes). The human genome encodes nearly 100 deubiquitinating enzymes, making them the largest family of enzymes in the ubiquitin system, which may give an indication of their importance. DUBs are responsible for ubiquitin precursor processing, ubiquitin recycling, trimming of ubiquitin chains, as well as having many other diverse roles in cellular function[3]. The activity of deubiquitinating enzymes has been implicated in several crucial pathways including cell growth and differentiation, development, DNA damage, disease pathways, transcriptional regulation and chromatin remodeling[4].

Deubiquitination enzymes can be divided into five families: ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), ovarian tumour proteases (OTUs), Machado-Joseph disease protein domain proteases (MJDs) and JAB1/MPN/MOV34 metalloproteases (JAMM)[3]. The first four families are classified as cysteine proteases and comprise the majority of deubiquitinating enzymes. The last family, JAMM, binds zinc and therefore are classified as metalloproteases. The cysteine hydrolases specifically cleave ubiquitin substrates with the general structure $Ub^{1\text{-}72}$-Leu^{73}-Arg^{74}-Gly^{75}-Gly^{76}-X, where X can be any small thiol, amine, ubiquitin molecule or even another protein[5].

USPs are the largest family of deubiquitination enzymes and they are also the group with the largest size variation (50-300 kDa). These high molecular weight deubiquitinating enzymes can process ubiquitin precursors, remove ubiquitin from protein conjugates and disassemble long ubiquitin chains. In contrast to the USPs, the UCHs are relatively small enzymes (<30 kDa) that catalyze the removal of peptides and small molecules from the C-terminus of ubiquitin. Most UCHs cannot generate monomeric ubiquitin from protein conjugates or disassemble polyubiquitin chains. There is growing evidence that the four known UCH enzymes (UCH-L1, UCH-L3, UCH37 and BRCA1-associated protein-1, BAP1) are involved in human oncogenesis[6].

One of the emerging functions of deubiquitination enzymes in the past two years has been histone modification and the regulation of chromatin. Enzymes such as USP3, USP7, USP16, USP21, USP22 and MYSM1 have been identified as being involved in these processes. Histone modification usually involves monoubiquitination. Condensation of chromosomes during metaphase is

accompanied by the deubiquitination of histones H2A and H2B. Knockdown of USP3, an enzyme that catalyzes the removal of ubiquitin from H2A and H2B, has been shown to delay S phase progression and mitotic entry[7], while knockdown of USP16, which deubiquitinates H2A, but not H2B, results in slow growth of HeLa cells due to defects in the mitotic phase of the cell cycle[8]. Deubiquitination of H2A was shown to be a prerequisite for phosphorylation of histone H3 serine 10 and chromosome segregation and also plays a critical role in *Hox* gene expression in *Xenopus laevis*[8].

The table below provides a sample of deubiquitination enzymes and their associated biological function.

Deubiquitination Enzyme	**Biological Function**
USP3, USP7, USP16, USP21, USP22, MYSM1, BRCC36	Chromatin remodeling
USP1, USP3, USP28	DNA damage
USP7, USP16, USP19, USP28, CYLD	Cell proliferation

DUB-Detector Assay

Despite the differences in their cellular roles and molecular sizes, the deubiquitinating enzymes all appear to hydrolyze their substrates through a common mechanism. Active Motif's DUB-Detector uses a universal ubiquitin substrate to detect enzymatic activity or to screen for potential inhibitors. The fluorescent substrate used in the assay is based on a C-terminal derivative of ubiquitin which is hydrolyzed by the cysteine protease class of enzymes to release a fluorescent signal proportional to the amount of enzyme activity. The fluorescence can be detected using a microplate reader with excitation of 485 nm and emission of 535 nm. For added convenience, the assay also includes HeLa nuclear extract and an inhibitor of all classes of deubiquitinating enzymes as positive and negative controls.

Kit Performance and Benefits

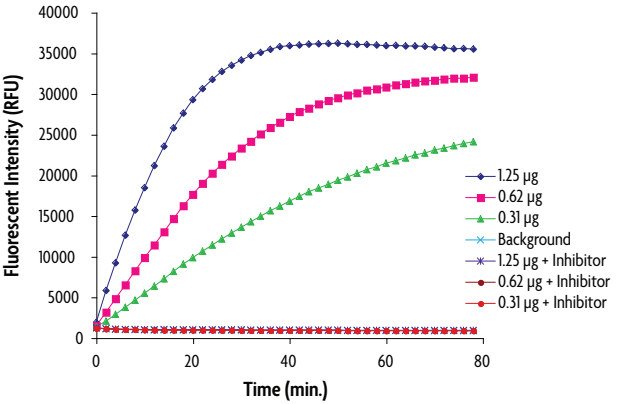
The DUB-Detector Deubiquitination Assay can be used to capture kinetic information about the deubiquitinating enzyme over a period of time, or end point data for a specified reaction time.

Range of detection: The assay can detect activity from recombinant proteins, purified enzymes, nuclear or whole-cell extracts. Recombinant proteins, purified enzymes and nuclear extracts may contain a higher concentration of deubiquitinating enzyme and therefore have a higher level of activity, while whole-cell extracts may hydrolyze the Fluorescent Substrate at a slower rate. To determine the optimal conditions for each sample, it is recommended initially to prepare a range of concentrations to determine the level enzymatic activity for each sample.

Cross-reactivity: The assay works with human, mouse and rat deubiquitinating enzymes. Other species have not been tested.

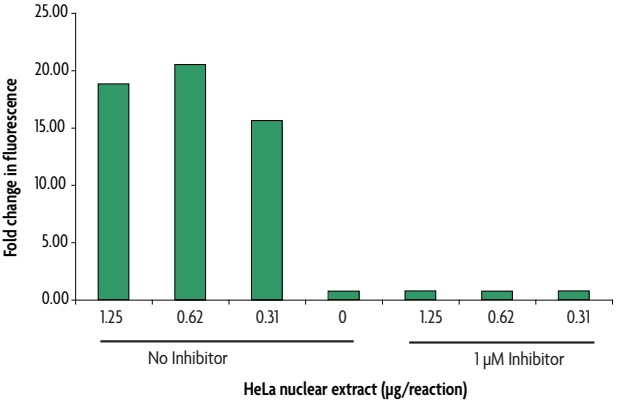
Assay time: 1.5 hours.

DUB-Detector™ Assay



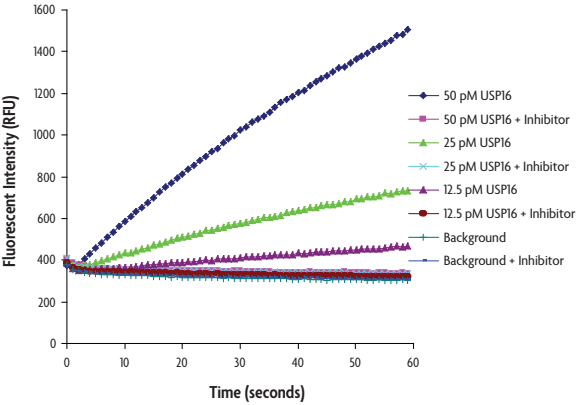
Kinetic Measurement of the deubiquitinating activity of HeLa nuclear extract.

The DUB-Detector™ Assay was used to assay activity of HeLa nuclear extract at 0.313, 0.625 and 1.25 µg per reaction. The extract was incubated for 20 minutes in the presence or absence of 1 µM Inhibitor. Following the incubation, 100 nM Fluorescent Substrate was added to each well and fluorescent intensity was immediately measured with an excitation wavelength of 485nm and an emission wavelength of 535nm. The fluorescent intensity of the reaction was measured every 2 minutes with a total reaction time of 80 minutes. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.



End point deubiquitinating activity of HeLa nuclear extract.

The DUB-Detector™ Assay was used to assay activity of HeLa nuclear extract at 0.313, 0.625 and 1.25 µg per reaction. The extract was incubated for 20 minutes in the presence or absence of 1 µM Inhibitor. Following the incubation, 100 nM Fluorescent Substrate was added to each well and the reaction proceeded for 60 minutes before the fluorescent intensity was measured with an excitation wavelength of 485nm and an emission wavelength of 535nm. The fold change in fluorescence was plotted. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.



Recombinant USP16 Deubiquitination activity.

The DUB-Detector™ Assay was used to assay activity of recombinant USP16 at 12.5, 25 and 50 pM per reaction. The enzyme was incubated for 20 minutes in the presence or absence of 100 nM inhibitor. Following the incubation, 100 nM Fluorescent Substrate was added to each well and fluorescent intensity was immediately measured with an excitation wavelength of 485nm and an emission wavelength of 535nm. The fluorescent intensity of the reaction was measured every minute with a total reaction time of 60 minutes. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.

Kit Components and Storage

DUB-Detector Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Fluorescent Substrate	15 µl	-80°C
Inhibitor	10 µl	-80°C
HeLa nuclear extract	100 µg (2.5 mg/ml)	-80°C
Assay Buffer AM2	15 ml	-20°C
1M DTT	2 x 100 µl	-20°C
Assay Plate	1	RT
Plate sealer	1	RT
Reservoir	1	RT

Additional materials required

- Deubiquitinating enzyme samples (recombinant, purified or nuclear extracted)
- Multi-channel pipettor capable of dispensing 10 µl volumes
- 1.5 ml microcentrifuge tubes for reagent dilution
- Aluminum foil
- Ice and ice bucket
- Timer
- Fluorescent microplate reader with filters capable of reading an excitation wavelength of 485 nm and an emission wavelength of 535 nm

Buffer Preparation and Recommendations

Complete Assay Buffer (See the Quick Chart for Preparing Buffers in this Section.)

Assay Buffer AM2 is provided frozen. To use, thaw the buffer at room temperature and the vial of 1M DTT on ice. Transfer the required amount of Assay Buffer AM2 for the number of wells being used to a separate tube. Prepare Complete Assay Buffer by adding 10 µl of 1 M DTT per ml of Assay Buffer AM2. We recommend adding the DTT immediately prior to use. Any remaining Complete Assay Buffer should be discarded.

Fluorescent Substrate (See the Quick Chart for Preparing Buffers in this Section.)

Do not leave the substrate at room temperature for long periods of time. It is recommended to prepare the Fluorescent Substrate during the designated incubation step within the protocol. Centrifuge the Fluorescent Substrate vial prior to opening to ensure complete recovery of the contents. The substrate is provided at a concentration of 50 µM in DMSO. Prepare a 500 nM working stock by making a 1:100 dilution of the fluorescent substrate into Complete Assay Buffer. Use 10 µl per well. The substrate is light sensitive and therefore should be protected from light whenever possible.

Note: It is important to avoid repeated freeze-thaw cycles of the substrate. We recommend making 5 µl aliquots of any unused substrate and storing at -80°C. Do not store substrate that has been diluted in assay buffer.

Inhibitor (See the Quick Chart for Preparing Buffers in this Section.)

Centrifuge the Inhibitor vial prior to opening to ensure complete recovery of the contents. The inhibitor is provided at a concentration of 50 µM. Prepare a 5 µM working stock by making a 1:10 dilution of Inhibitor into Complete Assay Buffer. Enough inhibitor is provided for 16 wells.

If screening potential inhibitor compounds, prepare inhibitor for use in a final volume of 10 µl per well.

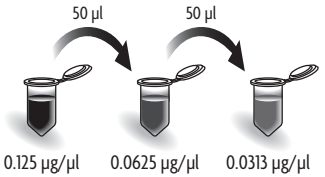
Samples

It is recommended initially to use a range of sample concentrations (*e.g.* 50 ng, 500 ng, 5 µg or 5 pM, 50 pM, 500 pM) in order to identify the optimal enzymatic conditions for your sample. Hydrolysis of the fluorescent substrate will occur very quickly in reactions containing extremely active, or high levels of deubiquitinating enzymes, which may cause the fluorescent response to take place before the plate is read. Preparing a range of sample concentrations will help to ensure the enzymatic reaction is within the detectable range. Samples should be diluted in Complete Assay Buffer and used in a final volume of 10 µl per reaction. (If no inhibitor is to be added to the samples, a final volume of 20 µl can be used. Total reaction volume should not exceed 50 µl.)

Once enzymatic activity has been determined within the assay, perform the rest of the assays within the optimized range. For samples with low enzymatic activity, incubations can be performed at room temperature or 30°C to allow the reaction to proceed more quickly.

HeLa Nuclear Extract (Also available separately as Catalog No. 36010.)
Unstimulated HeLa nuclear extract is provided as a positive control. The extract contains sufficient deubiquitinating activity when used between 0.3 - 1.25 µg per well.

To prepare the HeLa nuclear extract, thaw the extract on ice. Use three microcentrifuge tubes to make serial dilutions of the nuclear extract. Pipette 95 µl of Complete Assay Buffer into the first tube. Add 50 µl of Complete Assay Buffer to the remaining two tubes. Set up a serial dilution using the following concentrations: 0.125, 0.0625 and 0.0313 µg/µl. To make the serial dilutions, add 5 µl of HeLa nuclear extract (2.5 mg/ml) to the 95 µl in the first tube. Mix tubes thoroughly before each transfer using a vortex. Next, pipet 50 µl of diluted extract into the second tube. Vortex to mix. Finally, pipette 50 µl of the second dilution into the third tube. Vortex to mix. Keep the extracts on ice until ready to use. 10 µl of each dilution will be used per well.



Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 well	For 1 column (8 wells)	For 6 columns (48 wells)	For 12 columns (96 wells)
Complete Assay Buffer*	1M DTT	3 µl	8 µl	35 µl	70 µl
	Assay Buffer AM2	297 µl	792 µl	3.5 ml	7 ml
	TOTAL REQUIRED	**300 µl**	**800 µl**	**3.5 ml**	**7 ml**
Inhibitor (Optional)	Inhibitor (50 µM)	1.2 µl	9 µl		
	Complete Assay Buffer	10.8 µl	81 µl		
	TOTAL REQUIRED	**12 µl**	**90 µl**		
Fluorescent Substrate	Fluorescent Substrate Stock (50 µM)	0.12 µl	1 µl	6 µl	12 µl
	Complete Assay Buffer	12 µl	99 µl	594 µl	1.2 ml
	TOTAL REQUIRED	**12 µl**	**100 µl**	**600 µl**	**1.2 ml**

* Volumes listed refer to the preparation of buffer needed to dilute the HeLa nuclear extract, Fluorescent substrate, Inhibitor and set up the enzymatic reaction.

Assay Protocol

Read the entire protocol before use.

Determine the appropriate number of microwells required for testing samples, controls and blanks in duplicate. Due to the sensitivity of the assay, for kinetic analysis it is strongly recommended to run a minimum of 48 wells per experiment to enable the use of multi-channel pipettors, ensuring uniform delivery of the Fluorescent Substrate and consistent detection of enzymatic activity between wells. Cover unused wells with a portion of the plate sealer while you perform the assay.

Prepare the Complete Assay Buffer and Inhibitor as described above in the section Buffer Preparation and Recommendations. Keep reagents on ice until ready to use. If using 48 wells or more, multi-channel pipettor reservoirs may be used for dispensing the Fluorescent Substrate.

DUB-Detector Protocol

High concentrations of deubiquitinating enzymes in nuclear extracts, or strong enzymatic activity of recombinant enzymes may cause hydrolysis of the fluorescent substrate to occur very rapidly, such that the fluorescent response takes place before the plate can be scanned. To avoid this, it is recommended to test the sample extract or protein initially using a range of concentrations in order to determine the optimal amount.

Below is a plate set-up example to prepare positive control reactions in duplicate using the HeLa nuclear extract in the presence or absence of inhibitor.

	Positive Control extract											
	With Inhibitor	Without Inhibitor										
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.25 µg	1.25 µg										
B	1.25 µg	1.25 µg										
C	0.625 µg	0.625 µg										
D	0.625 µg	0.625 µg										
E	0.313 µg	0.313 µg										
F	0.313 µg	0.313 µg										
G	0 µg	0 µg										
H	0 µg	0 µg										

Step 1: Sample Preparation and Pre-incubation

1. Prepare samples and positive controls as described in the section Buffer Preparation and Recommendations. Pre-chill reagents and assay plate on ice.
2. Using the table below, add the reagents to each well of the plate in the order listed below, while keeping the plate on ice. Always change tips after adding inhibitor.

Reagents	Blank wells	Sample no inhibitor	(Optional) Sample with inhibitor	(Optional) Positive Ctl. no inhibitor	(Optional) Positive Ctl. with inhibitor
Complete Assay Buffer	40 µl	30 µl	20 µl	30 µl	20 µl
Inhibitor (Optional)	–	–	10 µl	–	10 µl
Sample	–	10 µl	10 µl	10 µl	10 µl

3. Cover the wells with plate sealer and incubate on ice for 20 minutes. This pre-incubation of the sample with DTT in the Complete Assay Buffer allows maximum enzymatic activity to be achieved. If the sample is known to have low enzymatic activity, the pre-incubation reaction can be performed at room temperature or 30°C.
4. During the incubation, prepare Fluorescent Substrate as described in the section Buffer Preparation and Recommendations. Keep the diluted Fluorescent Substrate protected from light. Also, prepare the settings for the fluorescent microplate reader.
   Excitation wavelength: 485 nm
   Emission wavelength: 535 nm.

Step 2: Addition of Fluorescent Substrate

DUB-Detector can be used to capture kinetic information about the deubiquitinating enzyme over a period of time, or end point data for a specified reaction time. Follow the protocol below for the desired analysis.

Kinetic Analysis

1. Remove the plate sealer. Scan the plate to establish a baseline fluorescent reading prior to the addition of the Fluorescent Substrate. This value can be used to blank the final readings.
2. Working quickly, manually add 10 µl of diluted Fluorescent Substrate to all wells. If adding substrate to several wells, it is recommended to add substrate to wells containing inhibitor first, then after changing tips, add substrate to the blank wells and samples without inhibitor.
   i. For manual delivery of the Fluorescent Substrate to 48 wells or more, the substrate can be added using a multichannel pipet and the provided reagent reservoir. Use a P-1000 pipet to transfer Fluorescent Substrate working solution into a thin line along the bottom of the reagent reservoir.
   ii. If less than 48 wells are to be analyzed, the Fluorescent Substrate needs to be delivered to each duplicate well and the plate scanned prior to adding Fluorescent Substrate to the next set of wells. Alternatively, the time difference between addition of Fluores-

cent Substrate to each sample set should be recorded and the plate scanned at an equivalent time for each sample. It is important to be as accurate as possible when recording the time for the addition of substrate as even small differences in timing will affect the final analysis of the enzymatic activity.

Note: The fluorescent substrate will begin to hydrolyze immediately, so it is important to perform this step as quickly as possible with the fluorescent microplate reader programmed and ready.

2. **I**mmediately insert the plate into the fluorescent microplate reader and start the scan. Continue to take readings for the desired length of time.

End Point Analysis

1. Remove the plate sealer. Scan the plate to establish a baseline fluorescent reading prior to the addition of the Fluorescent Substrate. This value can be used to blank the final readings.
2. Working quickly, add 10 µl of diluted Fluorescent Substrate to all wells. If adding substrate to several wells, it is recommended to add substrate to wells containing inhibitor first, then after changing tips, add substrate to the blank wells and samples without inhibitor.
   i. For manual delivery of the Fluorescent Substrate to 48 wells or more, the substrate can be added using a multichannel pipet and the provided reagent reservoir. Use a P-1000 pipet to transfer Fluorescent Substrate working solution into a thin line along the bottom of the reagent reservoir.
   ii. If less than 48 wells are to be analyzed, the Fluorescent Substrate needs to be quickly delivered with a single channel pipet.

   Note: The fluorescent substrate will begin to hydrolyze immediately, so it is important to perform this step as quickly as possible with the fluorescent microplate reader programmed and ready.

3. Incubate the reaction for the desired amount of time. Protect the plate from light during this time by placing it inside the microplate reader or by covering the plate with aluminum foil. If the enzymatic activity of the samples is unknown it is recommended to take multiple readings (*e.g.* 10 min., 30 min., 60 min.) to determine if the reaction has reached maximum activity. When ready, insert the plate into the fluorescent microplate reader and start the scan.

Calculation of fold change in fluorescence

To determine the fold change in fluorescence, average the duplicate readings from the baseline scan taken before the addition of substrate. Also average the duplicate readings from the fluorescent scan in the presence of substrate.

The fold change is determined by dividing the fluorescence values for each sample by the baseline values for the same sample. The fold change indicates the increase in fluorescent intensity as a result of the addition of the ubiquitin substrate and the activity of the deubiquitination enzymes.

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Appendix

Section A: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells in the correct order
	Substrate is no longer active	Avoid repeated freeze/thaw cycles of the Fluorescent Substrate. It is recommended to make small aliquots and store at -80C.
	Enzyme inhibitor present	The provided Inhibitor is very potent in very small amounts. It is important to change tips after addition of Inhibitor to each well.
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader. If the plate reader has a gain setting, check that it is set to a suitable level, usually between 60 and 120.
	Incorrect assay temperature	Enzymatic reactions can be performed in the range of 4C-37C. For samples with low enzymatic activity we recommend increasing the reaction temperature to at least 30C. Alternately, reaction time can be increased..
High fluorescent signal in sample wells	Concentration of deubiquitination enzyme is too high	If the sample being tested is very active, it is possible that it will hydrolyze the substrate within seconds, meaning the signal will be very high by the time the plate is scanned. It is recommended initially to test a range of sample concentration to determine the optimal amount for use in the assay.
Can a liquid injector system be used to deliver the Fluorescent Substrate to the plate?		If using a liquid injection system on a microplate reader, please verify the machine is capable of delivering 10 µl volume per well. Only a limited amount of substrate is provided in each kit. Verify that the amount of substrate provided in the kit, once diluted, is a sufficient volume for the plate reader to accurately deliver. Follow the instructions of the microplate reader to dispense Fluorescent Substrate into each well.
Can the assay be adapted for testing the zinc metalloprotease class of deubiquitination enzymes?		Zinc metalloproteases will require the addition of zinc to the assay buffer.

Section B. Related Products

Histone ELISAs	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110

Recombinant Methylated Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Control Acid Extracts	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium Butyrate treated)	100 µg	36202
HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

Histone Purification	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026
HeLa Core Histones	36 µg	53501

Co-Immunoprecipitation	Format	Catalog No.
Universal Magnetic Co-IP Kit	25 rxns	54002
Nuclear Complex Co-IP Kit	50 rxns	54001

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Shearing Kit	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Salmon Sperm DNA/Protein G agarose	25 rxns	53003
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

Fluorescent Dyes	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Carboxylic Acid	488 nm / 517 nm	1 mg	15510
Chromeo™ 488 NHS-Ester	488 nm / 517 nm	1 mg	15511
Chromeo™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromeo™ 494 Carboxylic Acid	494 nm / 628 nm	1 mg	15110
Chromeo™ 494 NHS-Ester	494 nm / 628 nm	1 mg	15111
Chromeo™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromeo™ 505 Carboxylic Acid	505 nm / 526 nm	1 mg	15610
Chromeo™ 505 NHS-Ester	505 nm / 526 nm	1 mg	15611
Chromeo™ 546 Carboxylic Acid	545 nm / 561 nm	1 mg	15210
Chromeo™ 546 NHS-Ester	545 nm / 561 nm	1 mg	15211
Chromeo™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromeo™ 642 Carboxylic Acid	642 nm / 660 nm	1 mg	15310
Chromeo™ 642 NHS-Ester	642 nm / 660 nm	1 mg	15311
Chromeo™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

Fluorescent Protein Labeling	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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