ab315074 – Human DDIT3 SimpleStep ELISA® Kit

For the quantitative measurement of DDIT3 in human and mouse cell extract.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab315074

Storage and Stability: Store kit at 2-8ºC immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
Human DDIT3 Capture Antibody 10X	600 µL	+4ºC
Human DDIT3 Detector Antibody 10X	600 µL	+4ºC
Human DDIT3 Lyophilized Recombinant Protein	2 Vials	+4ºC
Antibody Diluent CPR2	6 mL	+4ºC
Cell Extraction Buffer PTR 5X	10 mL	+4ºC
Cell Extraction Enhancer Solution 50X	1 mL	+4ºC
Sample Diluent NS	12 mL	+4ºC
Wash Buffer PT 10X	20 mL	+4ºC
TMB Development Solution	12 mL	+4ºC
Stop Solution	12 mL	+4ºC
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4ºC
Plate Seal	1	+4ºC

Sample Diluent NS is provided but not necessary for this product.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPR2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPR2. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the DDIT3 standard sample by adding the volume of 1X Cell Extraction Buffer PTR indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 40,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 375 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	25	375	40,000	2,500
2	Standard#1	150	150	2,500	1,250
3	Standard#2	150	150	1,250	625
4	Standard#3	150	150	625	312.50
5	Standard#4	150	150	312.50	156.25
6	Standard#5	150	150	156.25	78.13
7	Standard#6	150	150	78.13	39.06
8	Blank Control	0	150	N/A	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
A549 Cell Extract	62.5 - 1000 µg/ml
PC-3 Cell Extract	250 - 1000 µg/ml
Tunicamycin Treated HeLa Cell Extract	62.5 - 1000 µg/ml
NIH/3T3 Cell Extract	250 - 1000 µg/ml
Tunicamycin Treated C2C12 Cell Extract	15.63 - 125 µg/ml

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at 2x10^{7} cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human DDIT3.

The standard protein in this kit is full length human DDIT3.

Native signal was detected in human and mouse cell extract sample types.

Serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, saliva, urine, milk, CSF samples have not been tested with this kit.

This kit is incompatible with tissue extract samples.

SPECIES REACTIVITY

Rat species reactivity was determined by measuring rat PC-12 cell extract samples at an extract load of 1,000 µg/mL.

No signal was observed.

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements

Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.051	0.063	0.057
39.06	0.110	0.113	0.112
78.13	0.162	0.162	0.162
156.25	0.273	0.264	0.269
312.50	0.512	0.491	0.501
625	0.896	0.881	0.888
1,250	1.663	1.640	1.652
2,500	3.183	3.086	3.134

Table 1. Example of human DDIT3 standard curve in 1X Cell Extraction Buffer PTR. The DDIT3 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 9.88 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of DDIT3 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
125 µg/mL A549 Cell Extract	94	92 - 96
500 µg/mL PC-3 Cell Extract	85	85 - 87
125 µg/mL Tunicamycin Treated HeLa Cell Extract	96	90 - 99
500 µg/mL NIH/3T3 Cell Extract	96	93 - 98
31.25 µg/mL Tunicamycin Treated C2C12 Cell Extract	105	100 - 111

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native DDIT3 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	1,000 µg/mL A549 Cell Extract	1,000 µg/mL PC-3 Cell Extract	1,000 µg/mL Tunicamycin Treated HeLa Cell Extract	1,000 µg/mL Untreated HeLa Cell Extract
Undiluted	pg/mL	1,441.5	427.2	2,199.5	89.4
	% Expected	100	100	100	100
2	pg/mL	769.9	205.9	1,118.1	50.1
	% Expected	107	96	102	112
4	pg/mL	397.8	94.2	616.3	ND
	% Expected	110	88	112	N/A
8	pg/mL	206.7	ND	335.4	ND
	% Expected	115	N/A	122	N/A
16	pg/mL	104.2	ND	174.1	ND
	% Expected	116	N/A	127	N/A

ND – Not Detected – below product dynamic range

Dilution Factor	Interpolated value	1,000 µg/mL NIH/3T3 Cell Extract	125 µg/mL Tunicamycin Treated C2C12 Cell Extract	1,000 µg/mL Untreated C2C12 Cell Extract
Undiluted	pg/mL	330.7	1,645.8	97.3
	% Expected	100	100	100
2	pg/mL	173.1	867.2	53.3
	% Expected	105	105	110
4	pg/mL	91.4	435.1	ND
	% Expected	111	106	N/A
8	pg/mL	ND	244.0	ND
	% Expected	N/A	119	N/A

ND – Not Detected – below product dynamic range

Precision

Mean coefficient of variations of interpolated values of DDIT3 from a single concentration of tunicamycin treated HeLa cell extract within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.07	6.35

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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