StressMarq Biosciences INC.

Discovery through partnership | Excellence through quality

StressXpress®

Ubiquitin Detection Kit

Catalog# SKT-131 (20 Assay Kit)

Capture and detection of ubiquitinated proteins

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Storage
SKC-131A	StressXpress® ubiquitin matrix (50% slurry)	400µL	4°C
SKC-131B	StressXpress® columns	20 tubes	Ambient
SKC-131C	StressXpress® collection tubes	20 tubes	Ambient
SKC-131D	Ubiquitin Antibody, Clone FK2 (HRP) [Also known as: Catalog No. SMC-214]	25µL	4°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

Please read these instructions carefully before beginning this assay.

Kit contains sufficient StressXpress® ubiquitin matrix to perform up to 20 assays. The reagents in this kit have been tested and formulated to work exclusively with StressMarq Biosciences Inc.'s StressXpress® Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone:	250-294-9065
Fax:	250-294-9025
E-Mail:	techsupport@stressmarq.com
Hours:	M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

Kit components should be stored at the stated temperatures to ensure stability and activity.

Materials Needed But Not Supplied

- Microfuge tubes (1.5mL)
- Lysis buffer - 50mM Tris-HCl, pH7.5, 150mM NaCl, 0.5% (v/v) NP-40, 1mM DTT, 0.1% (v/v) protease inhibitor cocktail III (Roche)*
- Wash buffer - 50mM Tris-HCl, pH7.5, 150mM NaCl, 0.5% (v/v) NP-40, 1mM DTT*
- Elution buffer (as appropriate)
    - SDS-PAGE sample loading buffer – WB analysis
    - 0.1% formic acid solution – proteomic analysis
- Target protein specific antibodies – WB analysis
- Reagents for Western blotting
- Optional – proteasome inhibitor, or epoxomicin
- Optional – DUB inhibitor

*These buffers are just suggestions; other brands can be used.

INTRODUCTION

Background

The covalent attachment of ubiquitin to proteins (ubiquitination) plays a fundamental role in the regulation of cellular function through biological events including cell cycle, differentiation, immune responses, DNA repair, chromatin structure, transcription, signal transduction, endocytosis, apoptosis and degradation by the proteasome, autophagy and lysosome systems. As such ubiquitin signalling and the processes it mediates are essential for the normal functioning of cells and its dysfunction has been implicated in wide range of diseases including cancer, neurodegeneration, cardiovascular and metabolic disorders (1,2).

The type of ubiquitin modification, (monoubiquitin, multiubiquitin, polyubiquitin), substrate protein lysine residue(s) modified and, in the case of polyubiquitination, the chain length and lysine linkage type control the function and fate of ubiquitinated proteins. In addition all ubiquitin mediated pathways also utilise specific ubiquitin receptors to facilitate their regulation (3,4).

Ubiquitination is achieved through three enzymatic steps. In an ATP-dependent process, the ubiquitin E1 activating enzyme catalyses the formation of a reactive thioester bond with ubiquitin, followed by its subsequent transfer to the active site cysteine of a ubiquitin E2 carrier protein. The selectivity of the ubiquitin cascade for a particular substrate protein relies on the interaction between the E2 conjugating enzyme (of which a cell contains relatively few) and an ubiquitin E3 ligase, of which over 600 have been identified to date. The specific E2-E3 pair required for ubiquitination of a particular substrate protein in vivo may also control the type, point and length / linkage (polyubiquitin) of the ubiquitin modification (5).

About This Assay

Capture and detect ubiquitinated proteins

- High capacity, high specificity ubiquitin binding matrix for superior performance
- Fast, convenient protein isolation using StressXpress® purification system
- Purify mono- and poly-ubiquitinated proteins, independent of chain linkage or length
- Identify and analyse captured proteins by Western blotting or proteomic methods

The StressXpress® ubiquitin kit facilitates the fast, effective capture and detection of ubiquitinated proteins from biological samples. The kit utilises a high capacity, high specificity ubiquitin binding matrix together with StressMarq Biosciences' easy-to-use StressXpress® purification system for less 'hands on time' and superior performance. Allows purification of mono- and poly-ubiquitinated proteins, independent of chain linkage or length, but not free ubiquitin. Highly adaptable, compatible with samples from a wide range of species and with a broad range of lysis buffers. Analysis by Western blotting or proteomic methods enables identification and assessment of ubiquitinated proteins of interest. Kit contains sufficient StressXpress® ubiquitin matrix to perform up to 20 assays.

Use this kit to

1. Capture and detect ubiquitinated proteins and free chains from cell lysates and tissue extracts
2. Demonstrate specific proteins are substrates for ubiquitin modification in vivo
3. Identify and characterise ubiquitin modified proteins by proteomic analysis
4. Investigate role of ubiquitin in particular signalling pathways

Assay Preparation

Samples

Recommend using 100-200µL lysate at 5mg/mL per assay as a starting point. Adjust lysate concentration with lysis buffer if required.

Lysis Buffer

- Assay compatible with a wide range of lysis buffers
- Avoid buffer components that cause protein denaturation, especially chaotropes such as urea
- Minimise use of reducing agents (e.g. DTT) and detergents where possible
- Suggested lysis buffer: 50mM Tris-HCl, pH7.5, 150mM NaCl, 0.5% (v/v) NP-40, 1mM DTT, 0.1% (v/v) protease inhibitor cocktail III (Roche)
- Optional – Include proteasome or DUB inhibitors in lysis buffer

Elution Buffer

Select appropriate elution buffer for intended method of analysis:

a) SDS-PAGE sample loading buffer – WB analysis

b) 0.1% formic acid – proteomic analysis

Control reaction (recommended)

Widely used cell lysates such as HeLa S100 cytosolic fraction can be used as positive controls to demonstrate binding assay working / components functional

Assay Optimisation

Optimal assay conditions for capture of ubiquitinated proteins from specific lysate samples must be determined by the user. Adjustment of the following parameters may facilitate this process:

- Sample volume, 100-500µL
- Sample concentration, 1-5mg/mL
- StressXpress® ubiquitin matrix volume, 10-20µL settled resin
- Assay time, 1-4 hours or overnight

1. Keep reaction components on ice throughout set-up
2. Take 'Input' sample for subsequent analysis
3. Include appropriate controls as required

ASSAY PROTOCOL

StressXpress® ubiquitin matrix Preparation

4. Resuspend the StressXpress® ubiquitin matrix by gentle inversion of the tube
5. Aliquot 20µL StressXpress® ubiquitin matrix suspension into required number of capped StressXpress® columns
6. Add 500µL Wash buffer to capped column
   - Mix for 1 minute
   - Remove base cap
   - Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
   - Discard flow through
7. Repeat matrix wash / collection at least twice

StressXpress® ubiquitin Assay

8. Add 100-200µL lysate to capped StressXpress® ubiquitin matrix column and mix by inversion
9. Incubate for 1 hour at 4ºC with rotary mixing
10. Uncap column base and place in a StressXpress® collection tube
11. Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
12. Remove flow through and retain as 'Unbound Fraction' for subsequent analysis if required
13. Replace column in collection tube
14. Wash matrix by adding 500µL Wash buffer to column
    - Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
    - Repeat twice

Elution of captured ubiquitinated proteins

15. For SDS-PAGE / Western blot analysis:
    - Add SDS-PAGE sample loading buffer to capped column and mix by inversion
    - Place column in microfuge tube

- Heat to 95ºC for 5 minutes
- Remove base cap
- Centrifuge at low speed (1000-5000 g, 1 minute) to collect eluted materials
- Analyse or store at -20ºC

16. For proteomic analysis:
    - Add 10 volumes (100µL) 0.1% formic acid to capped column
    - Rotary mix for 5-10 at room temperature
    - Uncap column base and place in microfuge tube
    - Centrifuge at low speed (1000-5000 g, 1 minute) to collect eluted materials
    - Elution fraction can then be lyophilised and resuspended in trypsin digestion or alternative buffer prior to subsequent processing / analysis, or stored at -20ºC

ANALYSIS

Western blot Analysis

The StressXpress® ubiquitin kit includes ubiquitin mouse monoclonal antibody (FK2) HRP-conjugate for analysis by Western blotting.

Variable	Recommendation
SDS-PAGE	10% gel
Samples for analysis	Input Unbound (optional) Elution
FK2 antibody, HRP-conjugate	1:1000 dilution
Secondary antibody	Not required
Target protein specific antibody (user supplied)	Western blotting conditions must be determined by the user and the antibody applied in conjunction with an appropriate secondary antibody

Western blot analysis

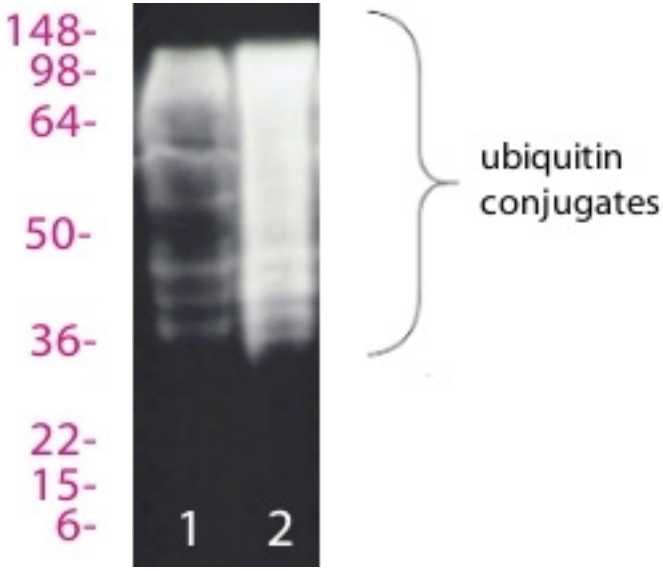

Figure 1: Western blot analysis of StressXpress® ubiquitin matrix capture of ubiquitin modified proteins from HeLa S100 lysate. Ubiquitin conjugates present in 'Input' (1) and Elution (2) samples were detected by ubiquitin monoclonal antibody FK2 (HRP) at 1:1000 dilution.

References

1. Fulda, S., Rajalingam, K. & Dikic, I. EMBO molecular medicine 4, 545–56 (2012). PMID: 22730341
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3. Husnjak, K. & Dikic, I. Annual review of biochemistry 81, 291–322 (2012). PMID: 22482907
4. Komander, D. & Rape, M. Annual Review of Biochemistry 81, 203–229 (2012). PMID: 22524316
5. Spasser, L. & Brik, A. Angewandte Chemie (International ed. in English) 51, 6840–62 (2012). PMID: 22696461

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Buyer's exclusive remedy and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

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NOTES

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