StressMarq Biosciences INC.

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StressXpress®

Ubiquitin Interact Detection Kit

Catalog# SKT-132 (20 Binding Assay Kit)

Capture and detection of ubiquitin binding proteins

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Storage
SKC-132A	StressXpress® ubiquitin interact matrix (50% slurry)	800µL	4°C
SKC-132B	StressXpress® columns	20 tubes	4°C
SKC-132C	StressXpress® collection tubes	20 tubes	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® Ub interact 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, 0.1% (v/v) Triton X-100, 5% glycerol, 0.1% (v/v) protease inhibitor cocktail III (Roche)
- Wash buffer - 50mM Tris-HCl, pH7.5, 150mM NaCl, 0.1% (v/v) Triton X-100, 5% glycerol
- 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

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 ubiquitin binding proteins

- Fast, convenient protein isolation using StressXpress® purification system
- Purify mono- and poly-ubiquitin binding proteins, independent of chain linkage or length

The StressXpress® ubiquitin interact kit facilitates the selective capture and detection of ubiquitin binding and associated proteins from cell lysates and tissue extracts. The kit utilises a high capacity, high specificity ubiquitin matrix together with StressMarq Biosciences' easy-to-use StressXpress® purification system for efficient isolation of ubiquitin binding proteins with minimal non-specific binding. Allows purification of mono- and poly-ubiquitin binding proteins, independent of chain linkage or length. Analysis by Western blotting or proteomic methods enables identification and assessment of captured proteins. Kit contains sufficient StressXpress® ubiquitin interact matrix to perform up to 20 binding assays.

Use this kit to

1. Identify and characterise isolated ubiquitin binding proteins by Western blotting or proteomic analysis
2. Demonstrate ubiquitin binding by specific proteins of interest
3. Investigate role of ubiquitin in particular signalling pathways

Assay Preparation

Samples

Recommend using 200µL sample / control 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
- **Note:** buffer used can influence identity of ubiquitin binding proteins captured – may require optimisation
- 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, 0.1% (v/v) Triton X-100, 5% glycerol, 0.1% (v/v) protease inhibitor cocktail III (Roche)

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

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 interact matrix volume, 10-20µL settled resin
- Assay time, 1-4 hours or overnight
- Lysis buffer composition

1. Keep reaction components on ice throughout set-up
2. Take 'Input' sample for subsequent analysis

StressXpress® ubiquitin interact matrix Preparation

3. Resuspend the StressXpress® ubiquitin interact matrix by gentle inversion of the tube
4. Aliquot 40µL StressXpress® ubiquitin interact matrix suspension into required number of capped StressXpress® columns
5. 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
6. Repeat matrix wash / collection at least twice

StressXpress® ubiquitin interact Assay

7. Add 200µL sample / control lysate to capped StressXpress® ubiquitin interact matrix column and mix by inversion
8. Incubate for 1 hour at 4°C with rotary mixing
9. Uncap column base and place in a StressXpress® collection tube
10. Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
11. Remove flow through and retain as 'Unbound Fraction' for subsequent analysis if required
12. Replace column in collection tube

13. 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

14. 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 95°C for 5 minutes
    - Remove base cap
    - Centrifuge at low speed (1000-5000 g, 1 minute) to collect eluted materials
    - Analyse or store at -20°C

Note: If required, pierce lid of spin tube prior to heating to prevent build-up of pressure

15. For proteomic analysis:
    - Add 10 volumes (200µL) 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 -20°C

Western blot Analysis

StressXpress® ubiquitin interact kit results can be analysed by Western blotting.

Variable	Recommendation
SDS-PAGE	10% gel
Samples for analysis	Input Unbound (optional) Elution
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

References

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3. Husnjak, K. & Dikic, I. Annual review of biochemistry 81, 291–322 (2012). PMID: 22482907
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5. Spasser, L. & Brik, A. Angewandte Chemie (International ed. in English) 51, 6840–62 (2012). PMID: 22696461

Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

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.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

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