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**StressXpress®**

## **Free Ubiquitin Chain Detection Kit**

Catalog# SKT-130 (10 Assay Kit)

Capture and detection of unanchored polyubiquitin chains

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## GENERAL INFORMATION

### Materials Supplied

Catalog Number	Reagent	Quantity	Storage
SKC-130A	StressXpress® free ubiquitin chain matrix (50% slurry)	400µL	4°C
SKC-130B	StressXpress® columns	10 tubes	Ambient
SKC-130C	StressXpress® collection tubes	10 tubes	Ambient
SKC-130D	Ubiquitin polyclonal antibody	25µL	-20°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® free ubiquitin chain matrix to perform up to 10 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**

<b>Phone:</b>	250-294-9065
<b>Fax:</b>	250-294-9025
<b>E-Mail:</b>	techsupport@stressmarq.com
<b>Hours:</b>	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. StressXpress® ubiquitin matrix should not be refrozen after initial thawing, store at 4°C.

## Materials Needed But Not Supplied

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

## Background

Ubiquitin typically exerts its fundamental role in the regulation of cellular function through covalent attachment of its C-terminal glycine (Gly<sup>76</sup>) to lysine residue(s) on substrate proteins (ubiquitination), often in the form of isopeptide linked polyubiquitin chains.

Non-substrate linked polyubiquitin chains, termed free or unanchored, have also been identified and been shown to play important roles in a range of cellular processes. Ubiquitin signalling using free ubiquitin chains is propagated via their non-covalent recognition by ubiquitin binding proteins. Processes involving free ubiquitin chains include: secondary messaging in NFκB signalling pathways (1,2); stress response (3); regulation of innate immunity (4); regulation of aggresomes (5,6); and as regulators of proteasome activity (7,8).

The origins of free ubiquitin chains have yet to be fully elucidated though specific ubiquitin E3 ligases (1,4) and deubiquitinating enzymes (DUBs) (5,9,10) are thought to play a role in their production.

As with polyubiquitin modified substrate proteins, the isopeptide linkages that connect the ubiquitin moieties of specific free ubiquitin chains are likely to define their particular function. Examples to date include: free K<sup>63</sup>-linked polyubiquitin chain activation of TAK1 kinase (1) and RIG-I (4); chains with linkages alternate to K<sup>63</sup> (and K<sup>48</sup>) in activation of the IKK complex (1); and free K<sup>48</sup>-linked ubiquitin chains in regulation of the 26S proteasome (8).

## About This Assay

Capture and detect unanchored polyubiquitin chains

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

The StressXpress® free ubiquitin chain kit facilitates the fast, effective capture and detection of unanchored polyubiquitin chains and free ubiquitin from biological samples. The kit utilises a high capacity binding matrix, specific for the free C-terminus of ubiquitin, together with StressMarq Biosciences' easy-to-use StressXpress® purification system for less 'hands on time' and superior performance.

Allows purification of free ubiquitin and free ubiquitin chains, independent of chain linkage or length. Highly adaptable assay, 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 analysis of free ubiquitin chains. Kit contains sufficient StressXpress® free Ub chain matrix to perform up to 10 assays.

## Use this kit to

1. Capture and detect free ubiquitin chains from cell lysates and tissue extracts
2. Identify and characterise free ubiquitin chains by proteomic methods
3. Assess effect of your experiment on free ubiquitin chain levels and composition
4. Investigate the physiological role of free ubiquitin chains

## PRE-ASSAY PREPARATION

### Assay Preparation

#### Samples

Recommend using 200 $\mu$ L sample / control lysate at 2.5mg/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
- Recommend inclusion of 5mM cysteine protease inhibitor N-ethylmaleimide (NEM)
- 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)
- Avoid buffer components that denature proteins, especially chaotropes such as urea
- Optional – Include proteasome or DUB inhibitors in lysis buffer

#### Sample Preparation (ESSENTIAL STEP)

1. Remove excess NEM from clarified lysate samples by incubation with DTT at 10mM final concentration for 15 minutes at 4°C
2. Heat NEM-quenched lysate to 75°C for 20 minutes
3. Centrifuge ( $\geq 15000$  g, 30minutes, 4°C) to remove any precipitate

This procedure denatures endogenous ubiquitin receptors that compete for binding to free ubiquitin chains whilst sparing the heat-stable chains leading to significantly increased recovery of chains and minimal ubiquitin receptor co-purification.

#### Elution Buffer

Select appropriate elution buffer for intended method of analysis, e.g. SDS-PAGE sample loading buffer for Western blotting.



## 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, 100-500 $\mu$ L
- Sample concentration, 1-5mg/mL
- StressXpress® free ubiquitin chain matrix volume, 10-30 $\mu$ L settled resin
- Assay time, 1-4 hours or overnight

## ASSAY PROTOCOL

1. Take 'Input' sample for subsequent analysis
2. Include appropriate controls as required

### StressXpress® free ubiquitin chain matrix Preparation

3. Resuspend the StressXpress® free ubiquitin chain matrix by gentle inversion of the tube
4. Aliquot 20µL StressXpress® free ubiquitin chain 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® free ubiquitin chain Assay

7. Add 200µL sample / control lysate to capped StressXpress® ubiquitin matrix column and mix by inversion
8. Incubate for 2 hours 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 500 $\mu$ L Wash buffer A to column
  - Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
  - Repeat twice
14. Remove detergent from matrix by adding 500 $\mu$ L Wash buffer B to column
  - Centrifuge at low speed (1000-5000 g, 1 minute) to collect matrix
  - Repeat twice

### Elution of captured unanchored polyubiquitin chains

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 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
16. For proteomic and other analyses:
  - Add appropriate volume of selected elution buffer 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 processed for subsequent analysis, or stored at -20°C

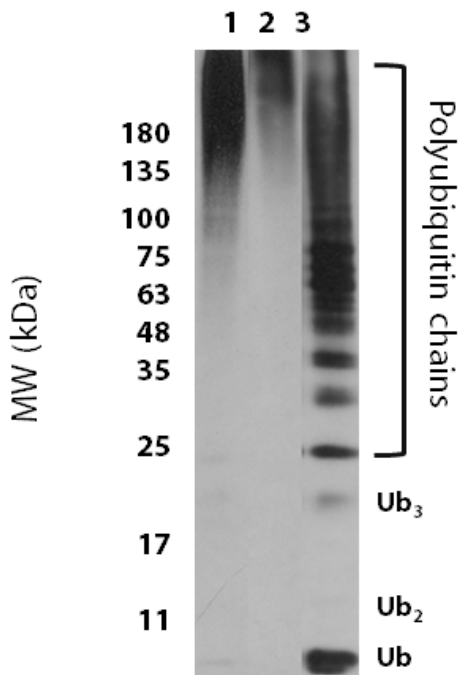
## ANALYSIS

### Western blot Analysis

The StressXpress® free ubiquitin chain kit includes ubiquitin rabbit polyclonal antibody for analysis by Western blotting.

Variable	Recommendation
SDS-PAGE	12% gel
Samples for analysis	Input Unbound (optional) Elution
Ubiquitin antibody	1:1000 dilution
Secondary antibody	Goat anti-rabbit (HRP-conjugate)

## Western blot analysis



**Figure 1:** Western blot analysis of StressXpress® free ubiquitin chain matrix capture of free ubiquitin and free ubiquitin chains from HEK293T lysate following heat treatment of samples. Ubiquitin species present in lysate sample before (1) and after (2) heat treatment and in elution fraction (3) were detected by ubiquitin polyclonal antibody at 1:1000 dilution.

### References

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## 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.**

## NOTES

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