Mono and Polyubiquitylated Conjugates mAb (clone FK2), HRP-linked

Ubiquitin Conjugate Antibody

Cat. No. **68-0122-100**
Lot. No. **30125**

Quantity: 100 µg
Storage: -20˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

Description

Anti-mono and polyubiquitylated conjugates mAb (FK2) demonstrates specific recognition for monoubiquitylated and polyubiquitylated proteins but shows no reactivity with free ubiquitin (Fujimoro *et al.* 1994). The anti-polyubiquitylated conjugates mAb (FK2) has been extensively characterised and used not only to investigate ubiquitin chain formation on poly and mono ubiquitylated proteins by Western blotting but also in the detection of intracellular polyubiquitin chains in immunoassays (Takada *et al.* 1995; Fujimoro *et al.* 2005). The HRP linked anti-mono and polyubiquitylated conjugates mAb (FK2) facilitates the detection of mono and polyubiquitylated ubiquitin conjugates by Western blotting without the use of a secondary antibody.

References:

Fujimuro M, Sawada H, Yokosawa H (1994) Production and characterization of monoclonal antibodies specific to multi-ubiquitin chains of polyubiquitinated proteins. *FEBS Lett* **349** 173-180.

Takada K, Nasu H, Hibi N, Tsukada Y, Ohkawa K, Fujimuro M, Sawada H, Yokosawa H (1995) Immunoassay for the quantification of intracellular multi-ubiquitin chains. *Eur J Biochem* **233** 42-47.

Fujimuro M, Yokosawa H (2005) Production of antipolyubiquitin monoclonal antibodies and their use for characterization and isolation of polyubiquitinated proteins. *Methods Enzymol* **399** 75-86.

Physical Characteristics

Clone: FK2

Isotype: IgM

Specificity: Recognises only monoubiquitin and polyubiquitin conjugates. Does not cross-react with free ubiquitin.

Molecular Weight: ~150 kDa

Immunogen: Crude preparation of polyubiquitylated lysozyme

Source/Host: BALB/c mouse implantation ascites

Quantity: 100 µg

Concentration: 1 mg/ml

Formulation: 10 mM phosphate buffer, 0.15 M NaCl pH 7.4, 0.1% sodium azide.

Stability/Storage: 12 months at -20˚C; aliquot as required

Quality Assurance

Anti-Mono and Polyubiquitylated Conjugates mAb (FK2) Biotin-linked Antibody Activity Assay: By Western blotting the specific recognition of mono and polyubiquitylated conjugates by the antibody over free ubiquitin was demonstrated (Figure 1).

A priming and extension assay was run containing, UBE1 [6His-tagged] (Cat# 61-0001), UBE2W [6His-tagged] (Cat# 62-0085), UBE2N [untagged] (Cat# 62-0047), UBE2V1 [untagged] (Cat# 62-0059), Ubiquitin (Cat# 60-0001), CHIP [untagged] (Cat# 63-0003) and ATP. Using the anti-mono and polyubiquitylated conjugates mAb (FK2) biotin-linked antibody, detection of polyubiquitin chains extending from mono-ubiquitylated CHIP (Lane 1) and free chains generated by UBE2N/UBE2V1 in the presence of CHIP (Lane 3) were observed. In the absence of CHIP, detection of free polyubiquitin chains generated by UBE2N/UBE2V1 (Lanes 5 and 7) and ubiquitylated E2 enzymes (Lanes 5 and 6) was observed (Figure 2).

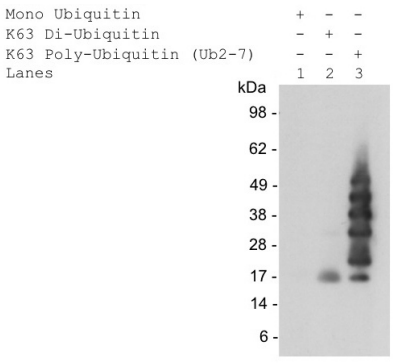

Figure 1

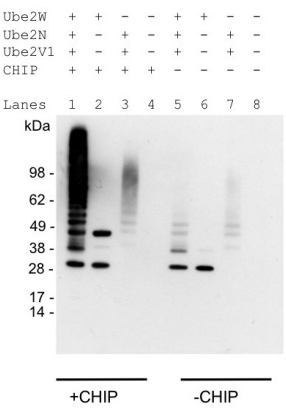

Figure 2

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Lot-specific COA version tracker: v1.0.0