Ubi*select*™-MEF_Bio-Ub (P0)

Cat. No. **66-5011-001**
Lot. No. **30347**

Quantity: $2.5x10^6$ cells
Storage: Cryopreserved

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The post-translational modification of proteins by ubiquitin is involved in a wide range of cellular processes (Kirkin and Dikic, 2007). In any given cell the proportion of protein modified by ubiquitin is very small therefore it has been difficult to isolate and identify this post translational modification from mammalian whole cell lysates. Ubiquitin proteomics remains challenging even though the sensitivity of Mass Spectrometry (MS) has improved dramatically through the use of innovative techniques (Sylvestersen *et al.*, 2013). Various approaches employing tagged ubiquitin or ubiquitin like molecules have been used with varying degrees of success (Peng *et al.*, 2003; Tirard *et al.*, 2012; Tsirigotis *et al.*, 2001). In recent years, the isolation of ubiquitylated proteins from neurons of *Drosophila melanogaster* using a tagged ubiquitin with a 15 amino acid long biotin-accepting peptide has been described (Franco *et al.*, 2011). This was the first demonstration that proteomics could be used to identify neuronal targets of the ubiquitin-proteasome system. This novel technique allowed for the isolation and enrichment of ubiquitin conjugates from neurons using a relatively small sample up to levels that allowed direct detection by MS and Western blotting. In addition, diglycine signatures indicative of the ubiquitin attachment sites could also be detected on ubiquitin conjugates. Where antibodies were available for specific ubiquitin proteasome system substrates it was also possible to determine whether a substrate was

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Culture Characteristics

Species: mouse

Source: mouse embryonic fibroblasts

Quantity: $2.5x10^6$ cells in 1 ml 90% fetal bovine serum (FBS), 10% DMSO

Culture Medium: DMEM high glucose (HG), 200 mM L-glutamine, 100 mM sodium pyruvate, 10% FBS, biotin 0.2 µg/mL, 1x pen/strep

Growth Mode: adherent

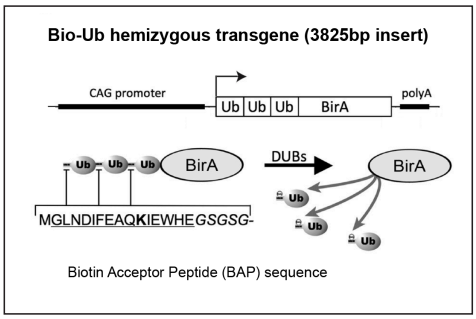
Passage Number: passage 0

Storage: cryopreserved

Culture Conditions: Thaw the cells by swirling the vial in a 37°C waterbath. Place 5 ml of culture media into a T25 flask and pipette cells into the flask containing the media. Place the T25 flask containing the cells into a 37°C 5% CO_2 incubator overnight and replace with fresh media the next morning.

Recommended sub-culture routine: When the Ubi*select*-MEF_Bio-Ub cells reach confluency, aspirate culture media from the T25 flask and rinse cells with 5 ml PBS. Aspirate the PBS from the T25 flask and add 1 ml 0.05% Trypsin-EDTA (1x). Incubate the BirA MEF cells in a 37°C 5% CO_2 incubator for a few minutes until the cells begin to detach, knock the side of the flask to fully detach the cells, neutralise with 4 ml of media and collect in a 15 ml tube then count the cells. Seed the Ubi*select*-MEF_Bio-Ub cells in T75 flasks at 1.2 x 10^4 cells/cm^2 (i.e. $9x10^5$ cells per T75 flask) and place in a 37°C 5% CO_2 incubator and grow until confluency (P1). Re-feed the Ubi*select*-MEF_Bio-Ub cells the morning after plating then every 3-4 days and passage as required until harvest.

Description of Transgene: This is a hemizygous cell line derived from a transgenic mouse expressing the *E. coli* enzyme BirA Accession number: [P06709.1] (Lectez *et al.*, 2014) and three copies of N-terminally BAP tagged ubiquitin.



Quality Assurance

Mycoplasma: Not detected

Morphology: Fibroblast

Viability (%): ≥95

Activity: For representative Ubi*select*-MEF_Bio-Ub (P0) cell line activity data, please refer to the Ubi*select*™ Kit application note (Application Note number 004).

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Lot-specific COA version tracker: v1.0.0

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mono- or polyubiquitylated (Franco *et al.*, 2011). Using a similar approach, a hemizygous Biotinylated-Ubiquitin (Bio-Ub) transgenic mouse has been created which expresses three moieties of ubiquitin each with an N-terminal Biotinylation Acceptor Peptide (BAP-tag) extension plus the *E. coli* enzyme BirA (Lectez *et al.*, 2014). The BirA enzyme recognises and biotinylates the BAP tag in the cell therefore enabling efficient capture of proteins ubiquitylated with this biotinylated ubiquitin. Ubi*select*-MEF_Bio-Ub (P0) (Cat# 66-5011-001) has been derived from 13.5 day old embryos of this transgenic mouse line by homogenisation and trypsinisation of embryos minus the head and liver. Ubi*select*-MEF_Bio-Ub (P0) may be used alongside the control Ubi*select*-MEF_BirA (P0) (Cat# 66-5010-001) in experiments to efficiently capture ubiquitylated proteins from treated and untreated MEF cells for analysis; see Application Note 004 for details. By using cell and tissue lysates derived from Bio-Ub transgenic mice it has been possible to isolate ubiquitylated proteins from ubiquitylated protein binding proteins (Lectez *et al.*, 2014). The specific isolation of ubiquitylated proteins is achieved through the high affinity interaction between biotin and avidin and high stringency washing which allows for the removal of interacting partners of ubiquitylated proteins which would otherwise predominate under the milder washing conditions of alternative ubiquitin capture systems. Using lysates prepared from Bio-Ub expressing liver tissue, the isolation of ubiquitin conjugated proteins (both thioester and isopeptide linked) has been demonstrated. Therefore this is the first mammalian system for ubiquitin proteomics which allows for the direct detection of captured proteins by MS and Western blotting. Isolation of ubiquitylated proteins from Ubi*select*-MEF_Bio-Ub (P0) has also been demonstrated (see Application Note 004) and it is anticipated that this technology will enable researchers to gain a better understanding of the functioning of ubiquitin system signalling.

References:

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Peng J, Schwartz D, Elias J E, Thoreen C C, Cheng D, Marsischky G, Roelofs J, Finley D, Gygi SP (2003) A proteomics approach to understanding protein ubiquitination. *Nat Biotechnol* **21**, 921-926.

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