

Epitope Mapping of EnCor Ubiquitin Monoclonal Antibody MCA-Ubi-1

Human	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
Macaque	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
Dog	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
Chicken	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
Danio	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
<i>Drosophila</i>	MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
<i>Caenorhabditis</i>	MQIFVKTLTGKTTITLEVEASDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60
<i>Arabidopsis</i>	MQIFVKTLTGKTTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLADY	60
<i>Saccharomyces</i>	MQIFVKTLTGKTTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDY	60

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<-----MCA-Ubi1----->
 | | | |
 30 <-----> 49
 GIPPDQQLI

Human	IQKESTLHLVLRIRGG	76
Macaque	IQKESTLHLVLRIRGG	76
Dog	IQKESTLHLVLRIRGG	76
Chicken	IQKESTLHLVLRIRGG	76
Danio	IQKESTLHLVLRIRGG	76
<i>Drosophila</i>	IQKESTLHLVLRIRGG	76
<i>Caenorhabditis</i>	IQKESTLHLVLRIRGG	76
<i>Arabidopsis</i>	IQKESTLHLVLRIRGG	76
<i>Saccharomyces</i>	IQKESTLHLVLRIRGG	76

Alignment of ubiquitin protein sequences from numerous species. Ubiquitin is one of the most conserved proteins known and is almost identical in protein sequence across species from human to yeast as shown above. Almost all cross species variation is conservative, meaning one amino acid is substituted for one of very similar properties. Epitope mapping was performed by generating a series of staggered 20 amino acid peptides which covered the human sequence with 5 amino acid overlap between neighboring peptides. Only the peptide, IQDKEGIPPDQQRLLIFAGKQ, amino acids 30-49, inhibited binding of MCA-Ubi-1 to purified bovine ubiquitin (highlighted above in yellow). Since the previous and next peptides had no apparent inhibitory effect on antibody binding, the central 10 amino acid segment, shown above in red, is likely the most significant component of the MCA-Ubi-1 epitope. This region corresponds to a turn followed by beta structure and is adjacent to Lysine 48, one of the sites at which ubiquitin may itself be ubiquitinated, producing poly-ubiquitin chains. The peptide is identical in all species shown here and in many other eukaryotes including representative animal, plant and fungal species suggesting that the Ubi-1 antibody is of unusually wide utility. We have obtained positive experimental results with cells and tissues originating from human, rat, mouse, cow, pig, dog and horse.