## Glutamate--cysteine ligase regulatory subunit

#### UniProt

<u>Pathway:</u> Sulfur metabolism; glutathione biosynthesis; glutathione from L-cysteine and L-glutamate: step 1/2.

<u>Tissue specificity:</u> In all tissues examined. Highest levels in skeletal muscle.

# Three antibodies: GCLM-1, GCLM-2 and GCLM-3 were tested. All three antibodies were approved for IHC. GCLM-1 was selected for full protein profiling.

### GCLM-1 (CAB040554)



#### Immunohistochemistry

**IHC protocol:** HIER pH 6, Dilution 1:15000 **IHC test staining:** Ubiquitous cytoplasmic staining with high expression in, eg skeletal muscle.

#### **IHC Annotators comments**

Most of the normal tissues displayed moderate cytoplasmic positivity. Additional nuclear staining was observed in squamous epithelia, gastrointestinal tract and urothelium. Alveolar cells, salivary gland, liver and breast glands were negative.

Moderate cytoplasmic immunoreactivity was observed in several cases of malignant lymphomas, melanomas, colorectal, cervical, endometrial and urothelial cancers. Additional nuclear staining was observed in squamous cell carcinomas, colorectal and urothelial cancers. A few cases of liver cancers exhibited strong staining. Other malignancies were in general weakly stained or negative.



### Immunofluorescence

**IF Overlay**: antibody (green), anti-tubuline (red) and DAPI (blue) **IF Localisation:** Staining of plasma membrane and cytoplasm in all three cell lines. Additional staining of nucleus in U-251 MG and A-431. Additional staining of focal adhesions in U-2 OS. **IF Validation:** Subcellular localization partly supported by literature or where no literature is available.



#### Western blot

WB Size markers (kDa): 250, 130, 95, 72, 55, 36, 28, 17, 11 WB Lanes: Marker(1), RT-4(2), U251 MG(3), Plasma(4), Liver(5), Tonsil(6) WB Target weight (kDa): 31 WB Validation: Supportive - Band of predicted size in kDa (+/-20%) with additional bands present.

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