|  |  |  |
| --- | --- | --- |
| **Samples** | **Total Ubiquitin** | **Free Ubiquitin** |
| **Mean of concentrations** | **Standard deviation** | **Group** | **Mean of concentrations** | **Standard deviation** | **Group** |
| **Developmental stages** |
| E0-3  | 0.35 | 0.07 | efg | 0.18 | 0.03 | de |
| E8-11  | 0.32 | 0.09 | fg | 0.18 | 0.03 | de |
| E16-19  | 0.35 | 0.11 | efg | 0.14 | 0.03 | e |
| L1  | 1.18 | 0.14 | a | 0.47 | 0.06 | b |
| L2 | 0.57 | 0.09 | cd | 0.26 | 0.09 | c |
| eL3 | 0.28 | 0.06 | fg | 0.15 | 0.03 | de |
| vL3 | 0.47 | 0.05 | de | 0.17 | 0.04 | de |
| P1  | 0.34 | 0.04 | efg | 0.23 | 0.03 | cd |
| P4 | 0.59 | 0.11 | c | 0.28 | 0.07 | c |
| P15 | 0.32 | 0.09 | fg | 0.17 | 0.04 | de |
| male0 | 0.41 | 0.08 | ef | 0.29 | 0.05 | c |
| male3 | 0.79 | 0.09 | g | 0.46 | 0.09 | de |
| female0 | 0.22 | 0.04 | b | 0.16 | 0.04 | b |
| female3 | 0.68 | 0.13 | c | 0.55 | 0.04 | a |
|  | **Tissues** |
| L3 brain | 0.29 | 0.02 | bc | 0.23 | 0.05 | b |
| L3 fatbody | 0.31 | 0.02 | bc | 0.11 | 0.04 | bc |
| salivary gland | 0.12 | 0.06 | c | 0.08 | 0.04 | c |
| testis | 0.35 | 0.19 | b | 0.23 | 0.09 | b |
| ovary | 0.61 | 0.19 | a | 0.38 | 0.05 | a |
| male head | 0.20 | 0.07 | bc | 0.12 | 0.05 | bc |
| male body | 0.21 | 0.05 | bc | 0.14 | 0.02 | bc |
| female head | 0.17 | 0.08 | bc | 0.13 | 0.05 | bc |
| female body | 0.66 | 0.22 | a | 0.42 | 0.18 | a |

**Table S1.** Multiple comparasion of the sample means.One-way ANOVA (p < 0.05) was performed, followed by SNK (Student–Newman–Keuls) post hoc test. Means indicated with the same letter are not significantly different.

|  |  |  |
| --- | --- | --- |
| **Buffer Composition** | **Mouse** | **Drosophila** |
| **Buffer F** | **Buffer T** | **Buffer F** | **Buffer T** |
| Tris  | 100 mM | 100 mM |
| NaCl | 20 mM | 150 mM |
| EDTA | --- | 1 mM |
| IAA | 10 mM | --- | --- |
| NEM | --- | 10 mM | --- |
| MG132 | 20 µM | 20 µM |
| DTT | --- | 2 mM | --- | 2 mM |
| CPIC | 1x | 1x |
| **pH** | 7.8 | 7.6 |
| **Incubation time at RT** | 2 h | 3 h |
| **Incubation temperature** | 37 °C | 25 °C |

**Table S2.** Optimal conditions for the ubiquitin quantification assay

EDTA= Ethylenediaminetetraacetic acid; IAA= Iodoacetamide; NEM= N-Ethylmaleimide; DTT= Dithiothreitol and CPIC= Complete Protease Inhibitor Cocktail