AdipoGen® LIFE SCIENCES

PRODUCT DATA SHEET

AG-20B-0020 15-Oct-2010

anti-Polyglutamylation Modification, mAb (GT335)

[Polyglutamylated Tubulin; Glutamylated Tubulin; Postranslational Protein Glutamylation]

AG-20B-0020-C100	100 µg
Clone	GT335
Source/Host	Purified from concentrated hybridoma tissue culture supernatant.
Isotype	Mouse IgG1κ
Immunogen	Octapeptide EGEGE*EEG, modified by the addition of two glutamyl units onto the fifth E (indicated by an asterisk).

Handling / Storage

Shipping	BLUE ICE
Short Term Storage	+4°C
Long Term Storage	-20°C

After opening, prepare aliquots and store at -20°C. Avoid freeze/thaw cycles.

Use / Stability

Stable for at least 1 year after receipt when stored at -20°C.

MSDS available at www.adipogen.com or upon request.

Product Specifications

Specificity	Recognizes the posttranslational modification (poly)glutamylation on proteins. Reacts with polyglutamylated α- and β-tubulin.
Species Crossreactivity	All
Application	**Electron Microscopy** (see Lit. 3) **Immunocytochemistry:** (1:2000) **Immunohistochemistry:** (paraffin sections; 1:1000) **Immunoprecipitation** **Western Blot:** (1:4000) Optimal conditions must be determined individually for each application.
Purity	≥95% (SDS-PAGE)
Formulation	Liquid. In PBS containing 0.02% sodium azide.
Concentration	1mg/ml
Isotype Negative Control	Mouse IgG1 Isotype Control

North/South America:
Adipogen Corp.
9853 Pacific Heights Blvd., Suite L
San Diego, CA 92121-4721
USA
TEL (858) 457-8383
FAX (858) 457-8484
info-us@adipogen.com

Rest of World:
Adipogen AG
Schützenstrasse 12
4410 Liestal
Switzerland
TEL +41-61-926-60-40
FAX +41-61-926-60-49
info@adipogen.com

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Other Product Data

Recognizes most forms of polyglutamylated tubulin (α- and β-tubulin), independent of the length of the glutamate side chains. No specificity to particular tubulin isoforms nor to tubulin from particular species are observed. Detects also other (poly)glutamylated proteins. Since no consensus modification site is known for protein (poly)glutamylation, the detection is not sequence-specific. However, an acidic environment of the modification site is required.

The use of the antibody at too high concentrations obscures its specificity in immunofluorescence.

Product Description

Polyglutamylation is a post-translational modification in which glutamate side chains of variable lengths are formed on the modified protein. It is evolutionarily conserved and the most prominent substrate is tubulin, the microtubule (MT) building block. Polyglutamylation has been proposed to be involved in the functional adaptation of MTs, as it occurs within the carboxy-terminal tubulin tails that participate directly in the binding of many structural and motor MT-associated proteins. The recent identification of new substrates of polyglutamylation indicates that this post-translational modification could be a potential regulator of diverse cellular processes and be involved in cell cycle and cell proliferation.

Product Specific References

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13. The actin-MRTF-SRF transcriptional circuit controls tubulin acetylation via α-TAT1 gene expression: J. Fernández-Barrera, et al.; J. Cell Biol. **217,** 929 (2018)

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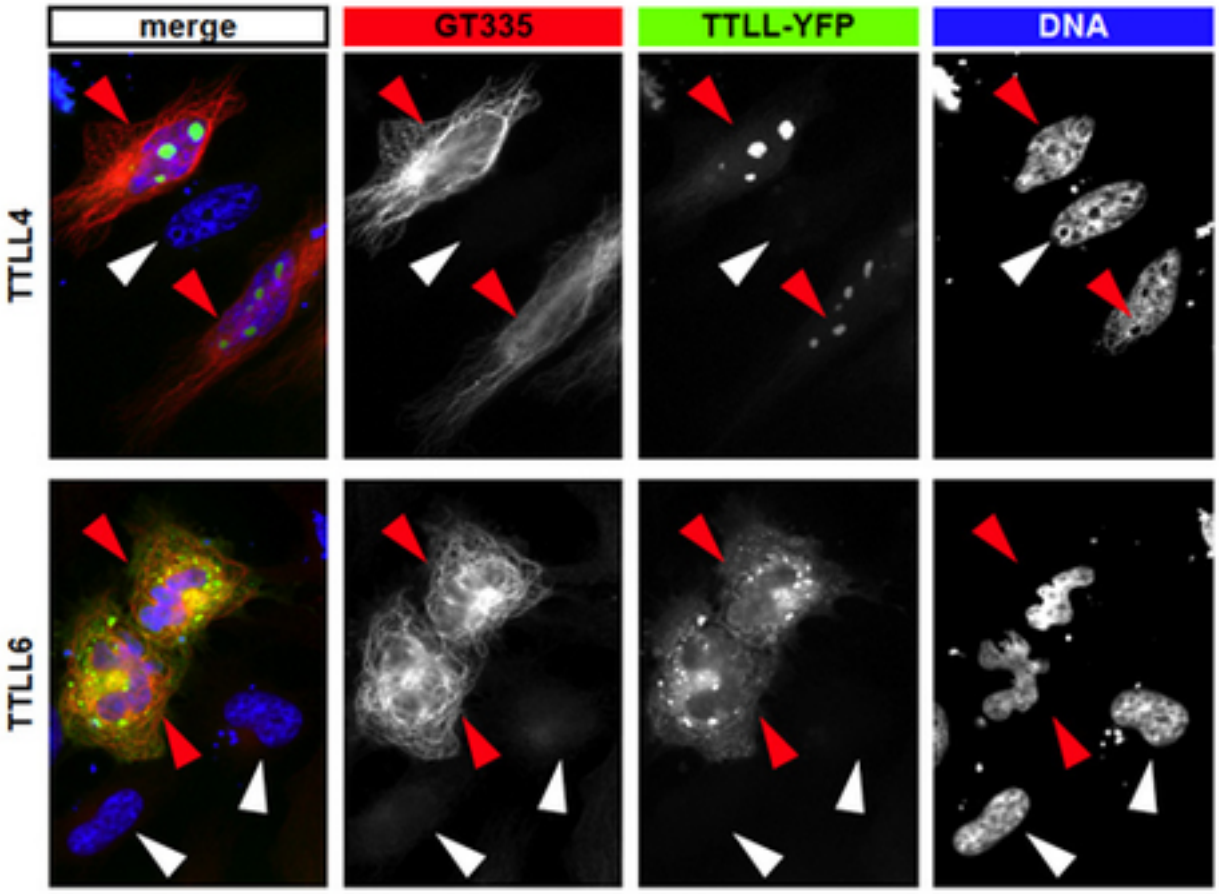

Figure 1: Immunofluorescence analysis of tubulin glutamylation with anti-Polyglutamylation Modification, mAb (GT335) (Prod. No. AG-20B-0020).
HeLa cells grown in standard culture conditions are transfected with GFP-fusion expression plasmids for the polyglutamylases TTLL4 and TTLL6. Glutamylated microtubules are detected with anti-Polyglutamylation Modification, mAb (GT335). Only in the cells expressing glutamylases (red arrowheads), strong microtubule labelling is detected, whereas in non-transfected cells (white arrowheads), only very weak labelling is seen. Note that the level of endogenous microtubule glutamylation can vary between different cell lines. *Picture courtesy of Dr. Carsten Janke, Curie Institute, Paris.*

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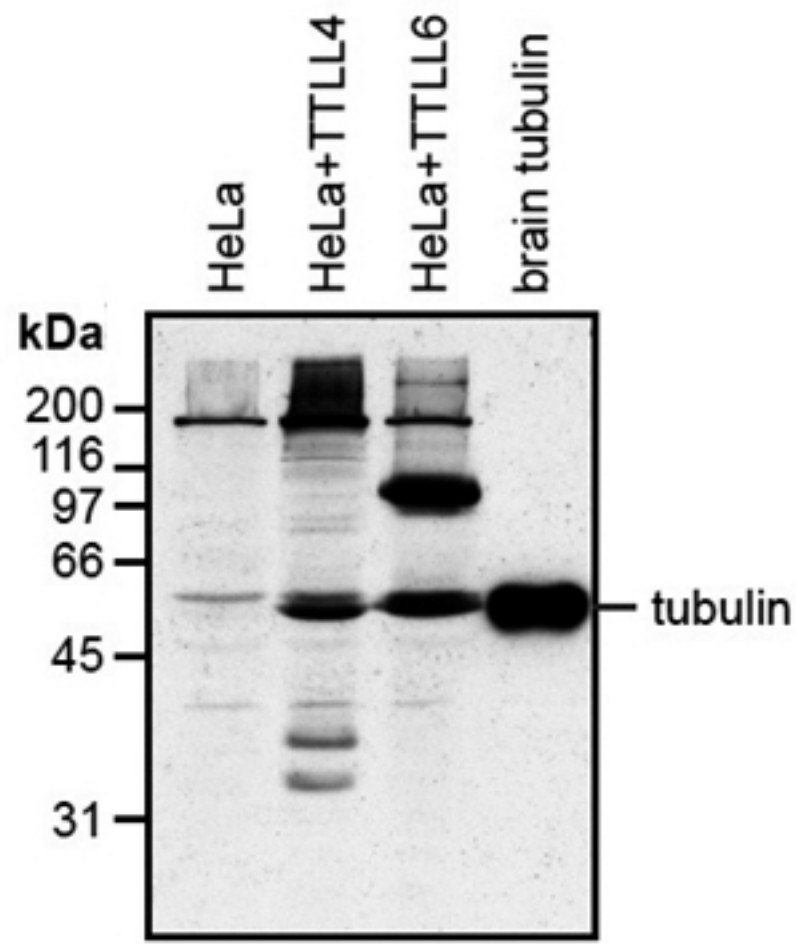

Figure 2: Western blot analysis of protein glutamylation with MAb to polyglutamylation modification (GT335) (Prod. No. AG-20B-0020).
Method: HeLa cells grown in standard culture conditions are lysed in SDS sample buffer and run on 10% SDS-PAGE. The proteins are transferred to nitrocellulose membrane and detected by standard Western blot protocol, using the MAb to polyglutamylation modification (GT335) (1:1'000) in TBS containing 0.1% Tween 20 for washing steps and 5% fat free milk for antibody incubation.
In HeLa cells, only two non-tubulin proteins are detected (Regnard et al., 2000; van Dijk et al., 2008), whereas after the expression of either TTLL4 or TTLL6 polyglutamylases (van Dijk et al., 2007), tubulin and additional proteins are detected. Brain tubulin is highly polyglutamylated and therefore strongly detected with the MAb to polyglutamylation modification (GT335).
Picture courtesy of Dr Carsten Janke, Curie Institute, Paris

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