Anti-Tet-Repressor Antibodies

Product Information Sheet
TET01, TET02, TET03

SUMMARY

shipped at RT; store at 4 °C

For research use only

Description

The tetracycline (tet) regulatory system is widely used for selective target gene regulation in eukaryotic cells. MoBiTec now offers a unique set of polyclonal and monoclonal antibodies targeting the Tet-Repressor protein (TetR-B) for study of this popular system. These antibodies possess excellent binding properties and have been successfully tested for use in ELISA, Western blot and immunofluorescence assays (not for IHC!). Two options for the monoclonal antibodies are offered. First an optimized mix consisting of two different epitope-specific monoclonal mouse antibodies (TET02), and second a single monoclonal mouse antibody, which can be used for immunofluorescence microscopy (TET03; not for IHC!). The rabbit polyclonal antibody (TET01) can be used in all three above-mentioned applications. These antibodies provide an excellent new tool for elucidating the tet regulatory system.

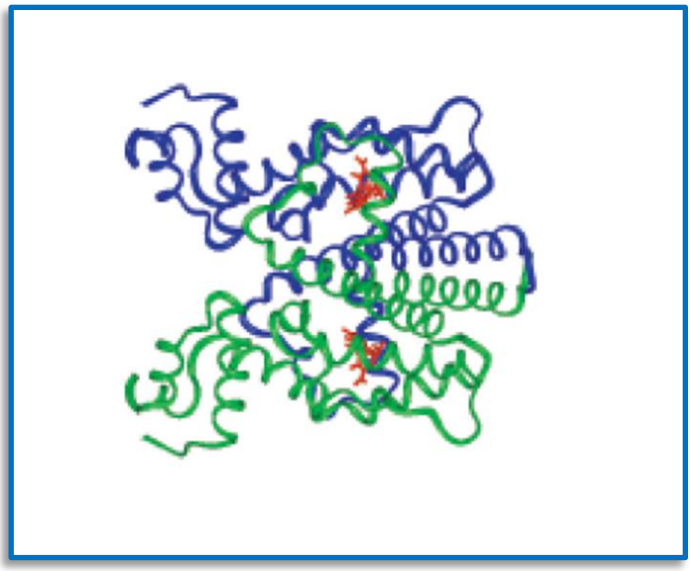

Fig.1: Structure of the Tet-Repressor (D)[tc•Mg]$^{+}$ complex.[7] The folding of the polypeptide chain is represented by a ribbon diagram. The subunits are shown in different colors. Illustration provided by Dr. E. Pook, formerly Institute of Microbiology and Biochemistry, University of Erlangen-Nürnberg, Germany.

NOTE: TET01-TET03 are suitable for immunofluorescence studies using cells. They do not work for tissues and tissue slices (IHC)!

Note:

MoBiTec anti-TetR(B) antibodies were raised against TetR(B)-tetO, but are generally able to detect also variants of TetR(B), like e.g. rtTA and its derivatives, whereby TET02 is the most sensitive and promising choice. However, in contrast to TetR(B), rtTA regulator proteins are required and present in cells in much lesser amounts, especially in stable cell lines. Additionally, the regulated gene and its product may downregulate these regulators further, so that it's even more obscured. That is, detection of rtTA and its derivatives is more difficult than that of TetR(B) and often fails at the level of Western blotting. In such cases, PCR may be used as surrogate due to its sensitivity. Nevertheless, independent references, examples see below, have proven the suitability of MoBiTec's anti-TetR(B) antibodies to also detect rtTA.

Nucleic Acids Res., Apr 2005; 33: e63
PLoS One. 2011;6(8):e23734
Transgenic Res (2012) 21:1099–1107
arXiv:1212.5109 [q-bio.MN] (2012)

Therefore, MoBiTec shall not be made responsible or liable for any claims or loss arising from the failure of the anti-TetR(B) antibodies to detect rtTA and its derivatives!

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Summary of the TetR(B) antibodies

	TET01	TET02	TET03
Type	Rabbit polyclonal IgG	Mouse monoclonal IgG1; K mix	Mouse monoclonal IgG1, K
Immunogen	TetR(B)-tetO	TetR(B)-tetO	TetR(B)-tetO
Purification	Affinity purified via Protein G columns	Affinity purified via Protein A or G columns	Affinity purified via Protein A or G columns
Epitope	___	TetR(B): Amino acid # 84 – 98 Amino acid # 26 - 53	TetR(B): Amino acid # 37 - 44
Reconstitution in	200 µl dest. H_2O	100 µl dest. H_2O	100 µl dest. H_2O
Working dilution for immunofluorescence	n.d.	n.d.	n.d.
Working dilution for Western blots and ELISA	1:1000	1:500 - 1:2000	1:1000
Detection limit ELISA	0.2 ng	20 - 50 pg	n.d.
Detection limit Western Blot	0.8 ng	3 ng	5 ng

Features

- Prime tool for studying tet regulatory systems in eukaryotic cells
- Suited for ELISA, Western blots and Immunofluorescence (for cells only, not for tissues or tissue slices)
- Excellent binding properties
- Monoclonal IgG1; K
- Polyclonal rabbit IgG
- Immunogen: TetR(B)-tetO
  (Accession no. PO4483)
- TetR(B) has a length of 207 amino acids and a mass of 23,355 Da
- TetR(B) is prone to form dimers.
  Also, formation of disulfide bridges is possible.
  Thus, multiple bands in WB may appear.

Perfectly suited for detection of:

- Tet-Repressor (TetR-B)Tet-Repressor
- Fusion protein (TetR-Fusion)
- Tetracycline responsive transactivator (tTA)
- reverse tetracycline responsive transactivator (rtTA) including derivatives like rtTA-S or rtTA-M (see note on p. 1)

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Examples of different application

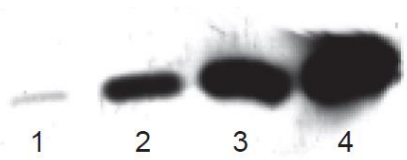

Fig.2: Western blot of polyclonal anti-TetR, diluted 1:1000, with different amounts of Tet-Repressor.
Lane 1: 0.8 ng; Lane 2: 4 ng TetR; Lane 3: 20 ng TetR; Lane 4: 100 ng TetR

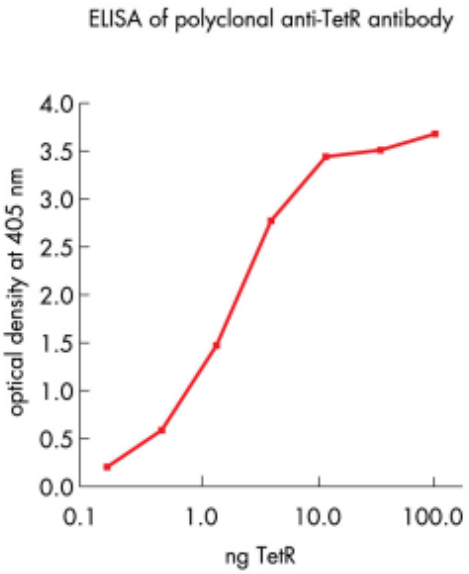

Fig.3: ELISA of polyclonal anti-TetR antibody. Wells were coated with TetR(B) overnight, blocked with 3% BSA and 0.05% Tween20 for 3 h at 37 °C, incubated with anti-TetR (diluted 1:1000) for 1 h at 37 °C, followed by 1 h at 37 °C with Protein A-alkaline-phosphatase. Substrate: 2 mg/ml para-nitrophenyl-phosphate in diethanolamine. Absorption measured at 405 nm.

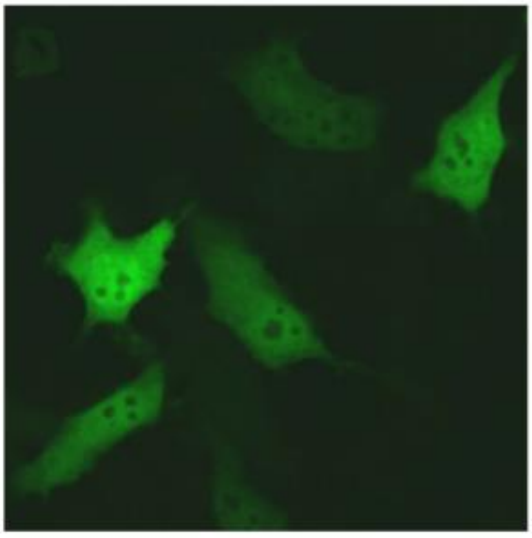

Fig.4: HeLa cells transfected with the plasmid pUHD15-1 (TetOFF). Monoclonal anti-TetR antibodies and secondary goat anti-mouse antibodies labeled with Alexa Fluor® 488 were used to stain TetOFF repressor protein.

Exemplary immunofluorescence protocol, according to Benabdellah et al., 2011 (27)

For immunofluorescence analysis of cultured cells, fix cells in 4% paraformaldehyde/PBS for 20 min, permeabilize with 0.1–1% Triton X-100/PBS for 15 min, and block with 5% PBS for 45 min at room temperature (RT). Incubate fixed cells with 2 µg/ml anti-Tet-repressor (TET02), followed by a secondary fluorophore-conjugated anti-mouse IgG. Mount stained cells in suitable mounting medium with DAPI (to stain the nuclei) and examine using a fluorescence microscope equipped with appropriate filters.

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Literature

Tet regulatory system:

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Order Information, Shipping and Storage

Order#	Product	Quantity
TET01	Anti-tet-repressor, polyclonal rabbit, lyophilized	3 mg
TET02	Anti-tet-repressor, monoclonal IgG1, mix, lyophilized	1 mg
TET03	Anti-tet-repressor, monoclonal IgG1, lyophilized	50 µg
shipped at RT; store at 4 °C		

Contact and Support

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