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.

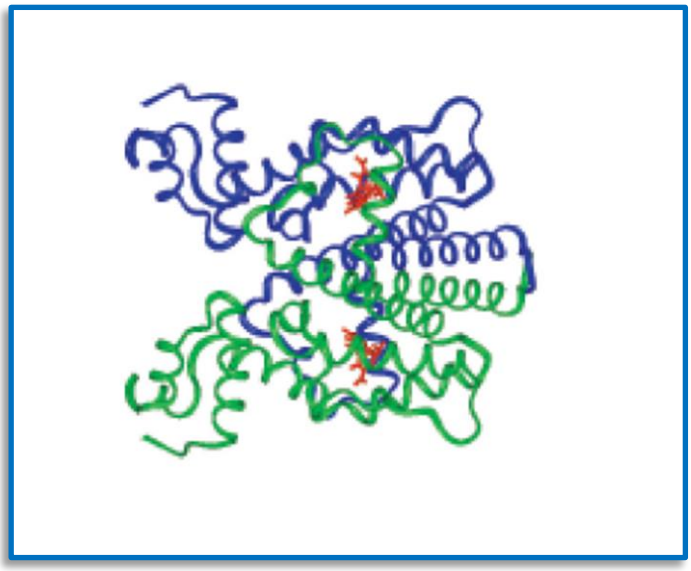

Fig.1: Structure of the Tet-Repressor (D)[tc•Mg]$^+$ complex.[7] The folding of the polypeptide chain is represented by a ribbon diagram. The subunits are shown in different colors. Illustration provided by Dr. E. Pook, formerly Institute of Microbiology and Biochemistry, University of Erlangen-Nürnberg,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

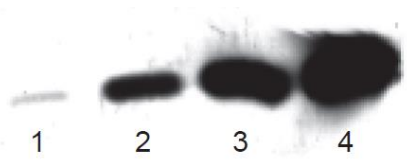

Fig.2: Western blot of polyclonal anti-TetR, diluted 1:1000, with different amounts of Tet-Repressor.
Lane 1: 0.8 ng; Lane 2: 4 ng TetR; Lane 3: 20 ng TetR; Lane 4: 100 ng TetR

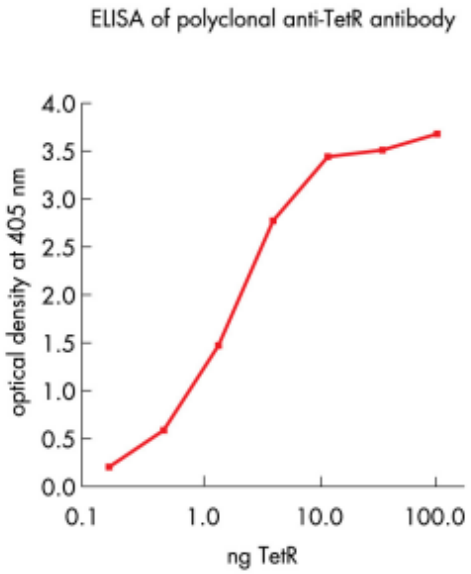

Fig.3: ELISA of polyclonal anti-TetR antibody. Wells were coated with TetR(B) overnight, blocked with 3% BSA and 0.05% Tween20 for 3 h at 37 °C, incubated with anti-TetR (diluted 1:1000) for 1 h at 37 °C, followed by 1 h at 37 °C with Protein A-alkaline-phosphatase. Substrate: 2 mg/ml para-nitrophenyl-phosphate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

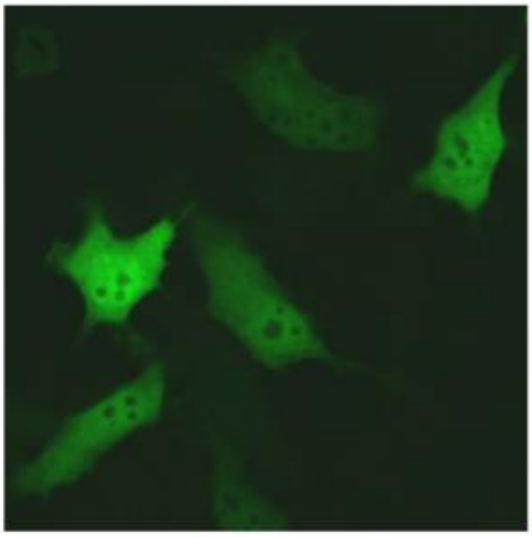

Fig.4: HeLa cells transfected with the plasmid pUHD15-1 (TetOFF). Monoclonal anti-TetR antibodies and secondary goat anti-mouse antibodies labeled with Alexa Fluor® 488 were used to stain TetOFF repressor protein.

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:

1. Pook E, Grimm S, Bonin A, Winkler T, Hillen W.(1998): Affinities of mAbs to Tet repressor complexed with operator or tetracycline suggest conformational changes associated with induction.
Eur J Biochem. 1998 Dec 15;258(3):915-22.

2. Hillen W, Berens C.(1994): Mechanisms underlying expression of Tn10 encoded tetracycline resistance.
Annu Rev Microbiol. 1994;48:345-69.

3. Gatz C.(1995): Novel inducible/repressible gene expression systems. Methods Cell Biol. 1995;50:411-24.

4. Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER.(1 996): Control of memory formation through regulated expression of a CaMKII transgene.
Science. 1996 Dec 6;274(5293):1678-83.

5. Hwang JJ, Scuric Z, Anderson WF. (1996): Novel retroviral vector transferring a suicide gene and a selectable marker gene with enhanced gene expression by using a t etracycline-responsive expression system.
J Virol. 1996 Nov;70(11):8138-41.

6. Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H. (1996): Transcriptional activation by tetracyclines in mammalian cells.
Science. 1995 Jun 23;268(5218):1766-9.

7. Hinrichs W, Kisker C, Duvel M, Muller A, Tovar K, Hillen W, Saenger W (1994): Structure of the Tet repressor-tetracycline complex and regulation of antibiotic resistance.
Science. 1994 Apr 1 5;264(51 57):41 8-20.

References with Tet antibodies

8. Marti F, Xu CW, Selvakumar A, Brent R, Dupont B, King PD. (1998): LCK-phosphorylated human killer cell-inhibitory receptors recruit and activate phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A. 1998 Sep 29;95(20):11810-5.

9. Freundlieb S, Schirra-Muller C, Bujard H.(1999): A tetracycline controlled activation/repression system with increased potential for gene transfer into mammalian cells.
J Gene Med. 1999 Jan-Feb;1 (1):4-12.

10. Urlinger S, Baron U, Thellmann M, Hasan MT, Bujard H, Hillen W. (2000): Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity.
Proc Natl Acad Sci U S A. 2000 Jul 5;97(14):7963-8.

11. Meissner M, Brecht S, Bujard H, Soldati D. (2001): Modulation of myosin A expression by a newly establishedtetracycline repressor-based inducible system in Toxoplasmagondii. Nucleic Acids Res. 2001 Nov 15;29(22):E115.

12. Lorenz P, Koczan D, Thiesen HJ (2001): Transcriptional repression mediated by the KRAB domain of the human C2H2 zinc finger protein Kox1/ZNF10 does not require histone deacetylation.
Biol Chem. 2001 Apr;382(4):637-44.

13. Fiedler M, Skerra A (2001): proBA complementation of an auxotrophic E. coli strain improves plasmid stability and expression yield during fermenter production of a recombinant antibody fragment.
Gene. 2001 Aug 22;274(1-2):111-8.

14. Stebbins MJ, Urlinger S, Byrne G, Bello B, Hillen W, Yin JC (2001): Tetracycline-inducible systems for Drosophila. Proc Natl Acad Sci U S A. 2001 Sep 11;98(19):10775-80. Epub 2001 Aug 21.

15. David KM, Perrot-Rechenmann C (2001): Characterization of a tobacco Bright Yellow 2 cell line expressing the tetracycline repressor at a high level for strict regulation of transgene expression.
Plant Physiol. 2001 Apr;1 25(4):1 548-53.

16. Reeves PJ, Callewaert N, Contreras R, Khorana HG (2002): Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line.
Proc Natl Acad Sci U S A. 2002 Oct 15;99(21):13419-24. Epub 2002 Oct 7.

17. Kamper MR, Gohla G, Schluter G (2002): A novel positive tetracycline-dependent transactivator (rtTA) variant with reduced background activity and enhanced activation potential. FEBS Lett. 2002 Apr 24;517(1-3):115-20.

18. Krueger C, Berens C, Schmidt A, Schnappinger D, Hillen W (2003): Single-chain Tet transregulators.
Nucleic Acids Res. 2003 Jun 15;31(12):3050-6.

19 Czauderna F, Santel A, Hinz M, Fechtner M, Durieux B, Fisch G, Leenders F, Arnold W, Giese K, Klippel A, Kaufmann J (2003): Inducible shRNA expression for application in a prostate cancer mouse model. Nucleic Acids Res. 2003 Nov 1;31(21):e127.

20 Leenders F, Mopert K, Schmiedeknecht A, Santel A, Czauderna F, Aleku M, Penschuck S, Dames S, Sternberger M, Rohl T, Wellmann A, Arnold W, Giese K, Kaufmann J, Klippel A (2004): PKN3 is required for malignant prostate cell growth downstream of activated PI 3-kinase.
EMBO J. 2004 Aug 18;23(16):3303-13. Epub 2004 Jul 29.

21 Das AT, Zhou X, Vink M, Klaver B, Verhoef K, Marzio G, Berkhout B (2004): Viral evolution as a tool to improve the tetracycline-regulated gene expression system. J Biol Chem. 2004 Apr 30;279(18):18776-82. Epub 2004 Feb 2.

22. Rupp B, Ruzsics Z, Sacher T, Koszinowski UH (2005): Conditional cytomegalovirus replication in vitro and in vivo. J Virol. 2005 Jan;79(1):486-94.

23. Markusic D, Oude-Elferink R, Das AT, Berkhout B, Seppen J. (2005): Comparison of single regulated lentiviral vectors with rtTA expression driven by an autoregulatory loop or a constitutive promoter. Nucleic Acids Res. 2005 Apr 4;33(6):e63.

24. Osada M, Park HL, Nagakawa Y, Yamashita K, Fomenkov A, Kim MS, Wu G, Nomoto S, Trink B, Sidransky D (2005): Differential recognition of response elements determines target gene specificity for p53 and p63. Mol Cell Biol. 2005 Jul;25(14):6077-89.

25. Ito T, Hashimoto Y, Tanaka E, Kan T, Tsunoda S, Sato F, Higashiyama M, Okumura T, Shimada Y (2006): An inducible short-hairpin RNA vector against osteopontin reduces metastatic potential of human esophageal squamous cell carcinoma in vitro and in vivo. Clin Cancer Res. 2006 Feb 15;12(4):1308-16.

26. Cai D, Byth KF, Shapiro GI (2006): AZ703, an imidazo[1,2- a]pyridine inhibitor of cyclin-dependent kinases 1 and 2, induces E2F-1-dependent apoptosis enhanced by depletion of cyclin-dependent kinase 9. Cancer Res. 2006 Jan 1;66(1):435-44.

27. Benabdellah K, Cobo M, Munoz P, Toscano MG, Martin F (2011): Development of an all-in-one lentiviral vector system based on the original TetR for the easy generation of Tet-ON cell lines. PLoS One, 2011 Aug 18; 6(8); e23734

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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		