# Product Data Sheet

# Rat anti-human Epo-R monoclonal antibody (GM-1201)

## Product information

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| **Catalog Number:** | GM-1201 |
| **Clone:** | BCO-3H2 |
| **Description:** | purified monoclonal rat antibody |
| **Specificity:** | anti-human Epo-R |
| **Isotype:** | IgG2b |
| **Purification:** | Protein G |
| **Storage:** | short term: 2°C - 8°C; long term: -20°C (avoid repeated freezing and thawing) |
| **Buffer:** | phosphate buffered saline, pH 7.2 |
| **Immunogen:** | peptide immunisation (GDSQGAQGGLSDGPYSN) from the cytoplasmic domain of human Epo-R |
| **Selection:** | based on recognition of the complete **native protein** expressed on mammalian cells and the **denatured protein** in Western blotting and immunohistochemistry |

## Working dilutions

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| **Flow cytometry only on permeabilised cells:** | 1.2 µg/$10^6$ cells |
| **ELISA:** | 1:200 - 1:400 |
| **CELISA only on permeabilised cells:** | 1:200 - 1:400 |
| **Western blotting:** | See ref. 5 |
| **Immunohistochemistry:** | See ref. 5 |

For each application a titration should be performed to determine the optimal concentration.

## Specificity testing by immunoprecipitation and Western blot

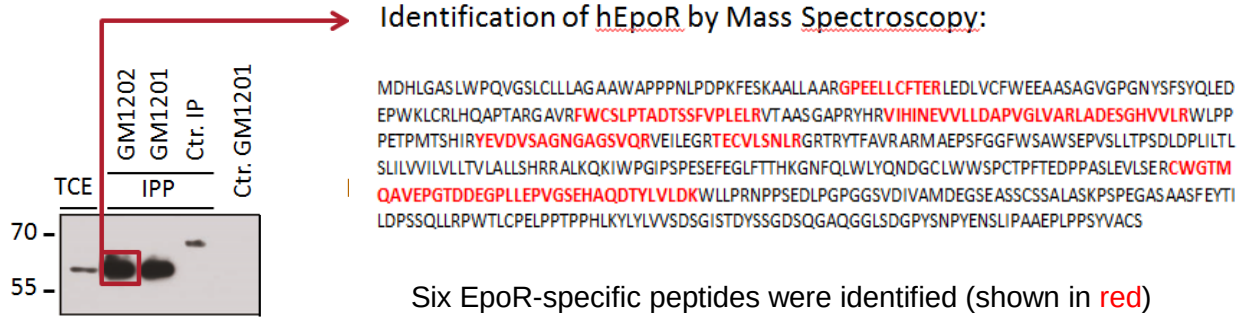


**Fig.1**: Mass spectroscopic analysis of EpoR immuno-precipitated from UT7 cells by GM-1201 or GM-1202 that were further analysed on denaturing SDS-PAGE gels (identified in Western blot with GM-1201). Segments of the gel were cut out for analysis by mass spectroscopy. For detailed information see Reference 5.

*For research use only. Not for diagnostic or therapeutic use.*

## CGE analysis of GM-1201

The antibody was purified by protein G affinity chromatography from cell culture supernatants and verified by CGE (Fig.2).

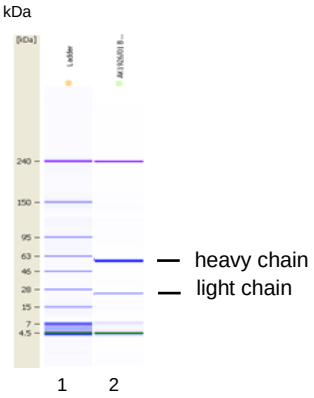


**Fig.2**: CGE analysis of purified GM1201 monoclonal antibody. Lane 1: molecular weight marker, Lane 2: 2 µg of purified GM1201 antibody. Proteins were separated by CGE (capillary gel electrophoresis, Agilent 2100 Bioanalyzer). Internal control bands (240 kDa / 7 kDa / 4,5 kDa).

## Background

Erythropoietin receptor (Epo-R) belongs to the cytokine receptor family and is a 507 amino acid type I transmembrane protein. EpoR pre-exists as dimers which changes the homodimerized state after binding of its 34 kDa ligand erythropoietin (Epo) (1,2). Erythropoietin is the primary regulator of erythropoiesis, and promotes the survival, proliferation, and differentiation of erythroid progenitor cells. Both, Epo and Epo-R are essential for the production of red blood cells due to Epo exerts its function through the Epo receptor (3). The Epo-R is also expressed in many organs outside the bone marrow, suggesting that Epo is a pleiotropic anti-apoptotic factor. Signaling pathways have been shown to influence numerous cellular functions in normal and tumor cells, including proliferation, apoptosis, and drug resistance (4). Development and specificity testing of the GM-1201 antibody are described in detail elsewhere (5).

## References


1. **Winkelmann JC (1992).** The human erythropoietin receptor. *Int J Cell Cloning*. Sep;10(5):254-61
2. **Livnah O, Stura EA, Middleton SA, Johnson DL, Jolliffe LK, Wilson IA (1999)**. Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. *Science* 283 (5404): 987–90
3. **Wilson IA, Jolliffe LK (1999)**. The structure, organization, activation and plasticity of the erythropoietin receptor. *Curr Opin Struct Biol.*;9(6): 696-704
4. **Hedley BD, Allan AL, Xenocostas A (2011)**. The role of erythropoietin and erythropoiesis-stimulating agents in tumor progression. *Clin Cancer Res*. 15; 17(20): 6373-80
5. **Maxwell P et al. (2015)**. Novel antibodies directed against the human erythropoietin receptor: creating a basis for clinical implementation. *Br J Haematol*; 168(3):429-42. doi: 10.1111/bjh.13133. Epub 2014 Oct 4