

## Humanized Anti-CD20/MS4A1 (Rituximab/Rituxan) Assay Kits

CD20 is a 33-36-kDa (297-aa) transmembrane phosphoprotein involved in the activation, proliferation, and differentiation of B-lymphocytes. It is absent in terminally differentiated plasma cells. It is alternatively known as MS4A1, membrane spanning 4-family sub domain A member 1, B-lymphocyte surface antigen B1, leu-16, Bp35, and CD-antige or CD20. Both N and C-termini located in the cytoplasm and 2 extra cellular regions: a large 44-amino acid loop (aa 142-184), which is the contact site of most anti-CD20 monoclonal antibodies (mAbs), including rituximab, and a small loop (aa 72-80), which is the contact site of human anti-CD20 mAbs. Rituximab also binds to amino acids 170-173 and 182-185 on CD20, which are physically close to each other as a result of a disulfide bond between amino acids 167 and 183. Significant levels of circulating CD20 (cCD20) can be detected in the plasma of CLL patients where they interfere with the binding of rituximab. The cCD20 levels correlated positively with beta-2-microglobulin level and percentage of CD38+ cells and negatively with platelet count and hemoglobin level. Circulating levels of Rituximab after a single dose vary among patients due to difference in tumor burden and clearance rates. Rituximab was detectable in the serum of patients three to six months after completion of treatment. Since Rituximab contains mouse antibody regions, it is more likely to induce antibodies than the humanized antibodies. According to the manufacture of Rituximab, less than 1% (3/355) of patients evaluated for human anti-chimeric antibody (HAMA/HACA) was positive. Availability of more sensitive ELISA for HAMA should be used to confirm the incidences of HAMA in rituximab-treated patients. HAMA/HACA titers may have allergic reactions when treated with this or other murine or chimeric monoclonal antibodies.

Rituximab destroys B cells, and is therefore used to treat diseases which are characterized by having too many B cells, overactive B cells or dysfunctional B cells. This includes many lymphomas, leukemias, transplant rejection and some autoimmune disorders. Rituximab has been shown to be an effective rheumatoid arthritis treatment. It is also used in autoimmune diseases such as hemolytic anemia, pure red cell aplasia, idiopathic thrombocytopenic purpura (ITP), Evans syndrome, vasculitis (for example Wegener's Granulomatosis), bullous skin disorders (for example pemphigus, pemphigoid—with very encouraging results of approximately 85% rapid recovery, type 1 diabetes mellitus, Sjogren's syndrome, and Devic's disease, and Graves' disease ophthalmopathy.

**Rituxan (rituximab)** is a genetically engineered chimeric murine/human monoclonal IgG1 kappa antibody (~145 kda). It is produced by mammalian cell (Chinese Hamster Ovary) suspension culture. The mouse/human chimeric CD20 mAb rituximab was the first cancer therapeutic mAb to be given Food and Drug Administration (FDA) approval and since then has become the most important new treatment for B cell malignancies in the last decade. The efficacy and success of Rituximab has led to some other anti-CD20 monoclonal antibodies being developed: **Ocrelizumab** (90%-95% humanized), **Ofatumumab** (HuMax-CD20, fully humanized), **Ibritumomab tiuxetan/Zevalin** (mouse mAb IgG1 in conjunction with the chelator tiuxetan) **Tositumomab/Bexxar** (mouse IgG2a is applied with iodine 131). Third-generation anti-CD20s have a glycol-engineered Fc fragment (Fc) with enhanced binding to Fc gamma receptors, which increase ADCC (antibody-dependent cellular cytotoxicity).



Rituximab destroys both normal and malignant B cells that have CD20 on their surfaces. Therefore, it may be advisable to carefully monitor patients for circulating CD20, drug concentrations during the treatments, and the development of antibodies (HAMA/HADA). ADI has developed new ELISA kits that measure **circulating CD20**, "**Free Rituximab** (CD20-unbound)" in patients treated with Rituximab. ADI has also developed ELISA kits to detect antibodies to Rituximab

(**Human Anti-Rituximab Antibodies**) in patients receiving long-term treatments. These kits will allow to research better ways to monitor rituximab treatment. Additional ELISA kits are available to monitor the increase other autoimmune diseases (ANA, anti-dsDNA IgGs, tuberculosis). ADI also have variety of antibodies to human, mouse, and rat CD20, and CD-20 peptides involved in rituximab binding.

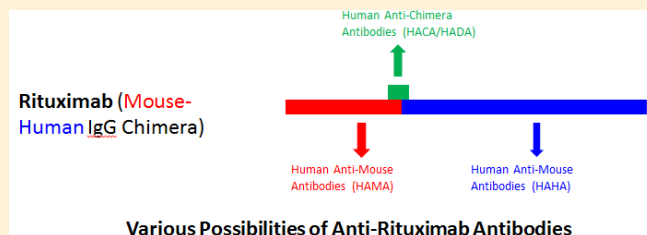
Catalog#	Product Description	Features
<b>200-210-RAG</b>	Rituximab/Rituxan (Active) ELISA Kit (Human/mouse/rat), 96 tests	Measures active (CD20-binding IgG) or free rituximab; This kit is suitable for mouse, rat, human etc.
<b>200-245-HAM</b>	Human Anti-Rituximab/Rituxan (HACA/HAMA/HAHA) IgG ELISA kit for human, 96 tests	Measures Anti-Rituximab IgG in human or animal samples. This test detect antibodies that may be directed to the mouse, human or chimeric region of rituximab (see details below).

- CD20-141-R **Recombinant Human CD20/MS4A1-mFC fusion Protein** (141-184, 44-aa, ECD, rituximab-binding region)
- CD20-145-R **Recombinant** (HEK cells) purified human CD20/MS4A1 (213-297 aa) his tag Protein
- CD20-146-R **Recombinant** (HEK cells) purified human CD20/MS4A1 (141-184 aa) his tag Protein
- CD20-147-R **Recombinant** (HEK cells) purified Ferret CD20/MS4A1 (213-297 aa) his tag Protein
- CD20-1731-P **Human CD20/MS4A1 peptide** (Acetyl-cPYaNPSLc, 9-aa, Cyclic Cys1-Cys9); contains ANPS motif and reactivity with Rituximab
- CD20-1732-P Human CD20/MS4A1 cyclic **peptide** (Acetyl-cWAANPSMAC, 11 aa, Cys1-Cys11); contains the ANPS motif and avidity for rituximab
- CD20-1733-P Human CD20/MS4A1 cyclic **peptide** (Acetyl-cPYsNPSLc; 9aa, Cys1-Cys9; contains NPS motif and react with rituximab
- CD20-21-M Monoclonal Anti-Human CD20/MS4A1 **peptide** (EC-domain, rituximab binding domain) IgG, ascites
- CD20-22-A Anti-Human CD20/MS4A1 **peptide** (EC-domain, rituximab binding domain) IgG, aff pure
- CD20-RP5L-P CD20/MS4A1 linear **peptide** (QDKLTQWPWKLEg, 13-aa) contains WPXWLE motif and reacts with rituximab
- CD20-142-P Human CD20/MS4A1 linear **peptide** (142-184, 43-aa, extracellular domain) rituximab-binding peptide, >95% pure
- CD20-1731-P Human CD20/MS4A1 **peptide** (Acetyl-cPYaNPSLc, 9-aa, Cyclic Cys1-Cys9); contains ANPS motif and reactivity with Rituximab

ADI is also offering custom testing of animal or human samples for humira or antibodies to humira, and TNF alpha measurements.

## Rituximab/Rituxan & Antibody ELISAs

All therapeutic antibodies, fully animal (mouse monoclonal) or fully human (humanized) or chimeric (mouse/rat-human IgG) must have specific test methods developed in order to measure their concentrations (CD20-binding or free form) in animal or human samples. This test is required for not only testing Rituximab during the manufacturing or storage process but also when administered into animal and patients. The assay can also be used to test the drug in its crude form (recombinant IgG expressed in CHO/HEK cell extracts) or in purified form. The type of assays that can be used or needed also depends upon the nature of the matrix or sample's environment. For examples, it may be possible to detect purified IgG by using simple A280 or by protein assay. The same assay will not work when detecting the crude IgG in CHO/HEK cells during its manufacturing. An assay to detect human IgG1 (or a relevant isotype of therapeutic antibody) will serve the purpose to measure purified human IgG1 (humanized IgG1) in the presence of other non-IgG proteins or non-human IgGs. But this IgG1 assay would be ineffective if measuring the therapeutic antibody (IgG1) in human serum containing endogenous IgGs including IgG1. In addition, a distinction should also be made on the basis of total antibody (total mass) Vs biologically active form, i.e. antigen-binding form or free active form. ADI has developed an ELISA to measure rituximab using the antigen-binding activity test (#200-210-RAG, see the details below). ADI also have an ELISA that measures the rituximab concn as as human IgG1 (not necessarily the active drug).

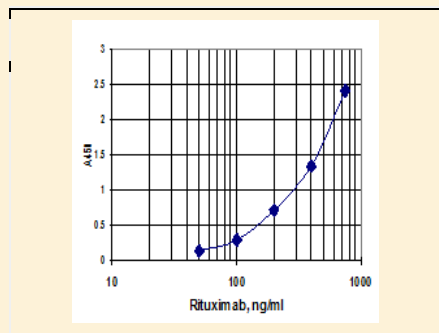


All antibodies, humanized or chimeric, have the potential to make antibodies even when injected into a homologous species (humanized antibody injected into human). Chimeric antibodies (e.g. Rituximab, mouse-human IgG) are expected to make anti-mouse antibodies when injected into humans (**HAMA or human anti-mouse antibodies**). There is also potential to make antibodies to the human portion of the rituximab (**HAHA or human anti-human antibodies**) due to change in conformation or structure of the chimeric IgG. There may even be antibodies that are directed against the mouse-human fusion regions or chimeric regions (**HACA or human anti-chimeric antibodies or Human Anti-Drug antibodies or HADA**). Therefore, test methods are needed to detect the production of HAMA, HAHA or

HACA/HADA. What kind of antibodies will be produced will also depend upon the species. For example, Rituximab when injected into human is likely to produce more HAMA or to the mouse portion whereas as mouse will mount a more robust response to the human portion or HAHA. ADI is the first company to develop new ELISA kits to detect and measure anti-rituximab antibodies (HAMA, HAHA, HACA/HADA) in mouse and human (#200-245-HAM, see the details below).. We also have other ELISA kits to measure either HAMA or HAHA in response to rituximab treatment.

### Rituximab/Rituxan (Active) ELISAs (Human Anti-CD20/MS4A1) ELISA Kit #200-210-RAG

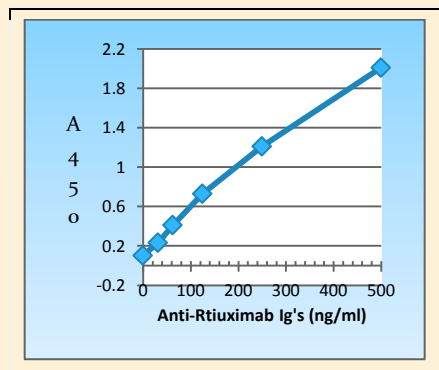
**This kit measures Active Rituximab (CD20-binding) in human or animal samples (buffers, serum or plasma).** It is a sandwich ELISA in which only active or non-liganded Rituximab from the sample is captured on the plate and then detected by antibody specific for Rituximab. No interference from endogenous IgGs from animal or human serum or plasma due to the proprietary design of the ELISA kit. For in vitro research use only.



- ELISA Kit Features**
- Active Rituximab/Rituxan binding, pre-coated, stabilized, ready-to-use 96-well strip plate, suitable for multiple runs over 6-12 months.
  - Active Rituximab IgG standards (0-750 ng/ml) and controls.
  - Sample size 100 ul (serum or plasma diluted ~1:100 or more).
  - 105 minutes, 3 incubation steps at room temp
  - Sensitivity ~25 ng/ml; Good Recovery and Assay Precision.
  - Contains all necessary reagents. Shelf life ~6 months.

### Human Anti-Rituximab IgG (HADA/HACA) ELISA Kit #200-245-HAM

This kit is designed to detect and measure antibodies to Rituximab (HADA or HACA) in humans. The test employs rituximab as capture antigen and the detection is by rituximab-enzyme conjugate. Therefore, this kit only detects antibodies (IgG or IgM etc) that are directed against the rituximab and not common HAMA or HAHA. The test will not distinguish if the antibodies are being to the mouse (HAMA) or human (HAHA) portion of the rituximab. This test can be used in human or mouse or other species. The test



- ELISA Kit Features**
- Rituximab, pre-coated, stabilized, ready-to-use 96-well strip plate, suitable for multiple runs over 6-12 months.
  - Anti-Rtx IgG standards (0-500 ng/ml)
  - Sample size 100 ul (serum or plasma diluted ~1:100 or more).
  - 105 minutes, 3 incubation steps at room temp
  - Sensitivity ~15 ng/ml; Good assay precision.
  - Contains all necessary reagents. Shelf life ~6 months.
- For in vitro research use only.
- Rev. 120912

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