

Malaria is a parasitic disease spread by mosquitoes. It affects about ~500 millions of people worldwide and killing an estimated 1 million annually. Over the last 50 years, vaccines for many debilitating diseases such as Diphtheria, Tetanus, Pertussis, Polio, Rabies, Mumps, Rubella, Tuberculosis, Hepatitis and Meningitis etc have been developed that are saving millions of lives worldwide. Despite many decades of research, an effective Malaria vaccine has remained elusive. The causative agent, the parasitic protozoan Plasmodium, is transmitted by mosquitoes. Four Plasmodium species infect humans. These are Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. Plasmodium berghei infects rodents. P. falciparum is the most widespread and also the most serious and potentially fatal form. The life cycle of the malaria is complex, with phases both in human host and the insect vector, the female anopheline mosquito. There are several Plasmodium forms: sporozoites, merozoites, gametocytes, gametes, ookinets, oocysts. Parasite may encode in the order of 2000 proteins, several hundred of which are antigenic. Proteins synthesized by each stage may be specific to that stage, such as liver stage-specific antigen (LSA-1), or be common to several stages, such as ring-infected erythrocyte surface antigen (RESA). The malaria parasite develops through several phases in the human body that evoke different immunologic responses, and vaccines for all phases are under development. The best-characterized protein of sporozoites is circumsporozoite protein-1 (CSP-1), an approximate 60 kDa protein located on the surface of developing and mature sporozoites and present in developing exoerythrocytic forms. It constitutes the major surface protein of the sporozoite. The central domain of CSP-1 is composed of an extensive array of tandemly repeated short sequences (NANP)<sub>n</sub> and (NVDP)<sub>n</sub>.



RTS,S is the most clinically advanced malaria vaccine candidate in the world today. It targets the pre-erythrocytic stage of the disease. RTS,S vaccine aims to induce antibodies to a parasitic protein (CSP-1) that is expressed in pre-erythrocytic stage and therefore prevent the parasite from infecting, maturing, and multiplying in the liver, and from re-entering the bloodstream and infecting red blood cells. RTS,S consists of two polypeptides that spontaneously form composite particulate structures on their simultaneous synthesis in yeast (*Saccharomyces cerevisiae*). RTS is a single polypeptide chain corresponding to CSP-1 amino acids 207-395 of *P. falciparum* (3D7) that is fused to HBsAg (adw serotype). S is a polypeptide of 226 amino acids that corresponds to HBsAg. The particles were purified from yeast-cell cultures and constitute the antigen used in the formulations. RTS,S is being developed by PATH, GlaxoSmithKline and the Bill and Melinda Gates

Foundation. The addition of GSK's proprietary Adjuvant Systems (AS01/AS02/QS21/MplA etc) aims to further improve the immune response. In October 2011, Phase III trial of RTS,S reported that it may protect approximately 50% of inoculated infants and children in malaria-endemic areas against infection and clinical disease caused by *Plasmodium falciparum*. No severe adverse events observed following the RTS,S vaccination were judged to be related to vaccination, though minor adverse events like headache, swelling, and malaise were.

Antibodies to the *Plasmodium falciparum* circumsporozoite repeat region were measured by ELISA using a recombinant antigen R32LR that contains the sequence [NVDP(NANP)<sub>15</sub>]<sub>2</sub>LR. Antibodies to HBsAg were also measured by ELISA. Antibodies to the CSP-1 protein are protective in animals, and in studies of infection in challenge models. Field trials show a relation between anti-CSP-1 antibody titres and re-infection rates after curative treatment with anti-malarials. However, no association between anti-circumsporozoite antibody titres and clinical malaria has been identified.

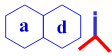
The primary objectives of a vaccine are to produce antibodies that will neutralize the causative agent. Therefore, it is extremely important to have simple, reliable, cost-effective methods to quantify not only the antibody titers but also the isotypes of antibodies and then correlate them with disease progression and vaccine efficacy. The main component of RTS,S malaria vaccine is the recombinant protein sequence encoded by CSP-1 protein (amino acids 207-395 of *P. falciparum* (3D7) that is fused to 226-aa HBsAg proteins). HBsAg not only serve as carrier protein for CSP-1 but there is added benefit of inducing antibodies to HBsAg and protection from hepatitis B. A review of the Phase II clinical data on the antigenicity of the RTS,S vaccine reveals that anti-CSP-1 antibodies were measured using an antigen, R32LR (recombinant or synthetic peptides containing (NANP)<sub>15</sub> or (NANP)<sub>30</sub>(NVDP)<sub>4</sub>-LR or about 65-130 peptide. This antigen is not the full-length CSP-1 that was actually used in the vaccine. The assumption made was that R32LR antigens detected antibodies from malaria samples or vaccinated animals. However, R32LR antigen is not a substitute for an extended protein used in the vaccine and the R32LR-antigen based ELISA may miss antibodies that will be directed against the epitopes not present in R32LR. The truncated antigen (R32LR) will also assume a different conformation or structure than the full length CSP-1 protein in the vaccine. It is strongly advisable to use full length CSP-1 protein that was actually used in the vaccine for antibody ELISA and to draw conclusions about the RTS,S antigenicity, malaria progression, and ultimately vaccine efficacy.

ADI is the first company to develop an antibody ELISA to determine the efficacy of the RTS,S vaccine. ADI's RTS,S antibody ELISAs (mouse, rabbit, and human) use the recombinant *P. falciparum* CSP-1 protein (207-395aa) that is the most critical and an active component of the RTS,S vaccine. ADI is further expanding the RTS,S antibody ELISAs to measure IgG (and IgG1, IgG2a, IgG3, IgG4) and IgM classes.

**Malaria Related Reagents and ELISA kits**

| Items Description  | Species | Antibody Type IgG Cat# | Antibody Type IgM Cat# |
|--|---------|------------------------|------------------------|
| <b>RTS,S Malaria Vaccine</b> (CSP-Antibody, <i>P. falciparum</i> ) ELISA Kits  | Mouse   | 970-300-MMG            | 970-310-MMM            |
|  | Rabbit  | 970-200-CSR            | 970-210-CSM            |
|  | Human   | 970-400-CHG            | 970-410-CHM            |
| <b>MSP, Malaria Vaccine</b> (MSP-1 Antibody, <i>P. falciparum</i> ) ELISA Kits | Mouse   | 970-320-MSG            | 970-330-MSM            |
|  | Rabbit  | 970-340-RMG            | 970-350-RMM            |
|  | Human   | 970-360-HMG            | 970-370-HMM            |

**Note:** ADI also developed antibody ELISA kits using (NANP)<sub>n</sub> and (NVDP)<sub>n</sub> synthetic peptides.



## Malaria Vaccines Antibody ELISA Kits, Recombinant Proteins, Peptides and Antibodies

| Item                     | Catalog#        | ProdDescription   | ProductType                   |
|--------------------------|-----------------|---|-------------------------------|
| CSP Protein and peptides | CSPF11-S        | Rabbit Anti-Circumsporozoite (CSP, P.falciparum) C-terminal (207-397 aa) protein antiserum  | Antibodies                    |
|                          | CSPF15-P        | YLKKIKNSL, P. falciparum circumsporozoite (CSP) peptide (CSP334-342)  | Peptides pure                 |
|                          | CSPF15-R        | Recombinant (E. coli, full length, CSP antigen (P. falciparum)  | Recomb. Protein               |
|                          | CSPF16-R        | Recombinant CSP mosaic protein (107-129, 334-351 aa) P.falciparum purified  | Recomb. Protein               |
|                          | CSPF17-R-10     | Recombinant (E. coli), purified, Circumsporozoite (CSP) (207-397 aa) P.falciparum Protein   | Recomb. Protein               |
|                          | CSPV11-M        | Mouse Anti-Circumsporozoite (CSP) (P. vivax) IgG, aff pure #1   | Antibodies                    |
|                          | CSPV16-R        | Recombinant (E. coli) CSP; 353-aa and GST) antigen (P. vivax)   | Recomb. Protein               |
|                          | CSPY11-P        | KIYNRNIVNRLDGD, P. yoelii circumsporozoite, PyCSP (57-70) peptide   | Peptides pure                 |
|                          | CSPY12-P        | SYVPSAEQI, P. yoelii circumsporozoite, PyCSP (280-288) peptide  | Peptides pure                 |
|                          | DRAA31-A        | Rabbit Anti-(DRAAGQPAG)3 peptide (repeat-sequence peptide of the P. vivax circumsporozoite protein, CSP) IgG, aff pure                                      | Antibodies                    |
|                          | DRAA31-BSA      | (DRAAGQPAG)3 peptide (repeat-sequence of the P. vivax CSP) conjugated with BSA  | Conj. Peptides                |
|                          | DRAA31-P        | (DRAAGQPAG)3 (repeat-sequence P. vivax CSP) control/blocking peptide  | Peptides pure                 |
|                          | DRADG31-P       | (DRADGQPAG)3 peptide (repeat-sequence peptide of the P. vivax CSP protein, pure   | Peptides pure                 |
|                          | Hemoglobin (hb) | RP-650  | Recombinant Malaria Cs Mosaic |
| HBG25-P                  |                 | DABCYL-GABA-ERMFLSFP-EDANS, Hb, 3037a, Malaria FRET Substrate II  | Substrates                    |
| HRP                      | HBG31-P         | DABCYL-GABA-ALERMFLSFP-EDANS, Hb, 2837a, Malaria FRET Substrate III   | Substrates                    |
|                          | HRPF21-M        | Mouse Anti-Histidine rich glycoprotein II (HRP II, P. falciparum) IgG, aff pure #1  | Antibodies                    |
| LSA                      | HRPF22-M        | Mouse Anti-Histidine rich glycoprotein II (HRP II, P. falciparum) IgM, aff pure   | Antibodies                    |
|                          | HRPF25-R        | Recombinant (E. coli) Histidine rich glycoprotein II (HRP II, P. falciparum)  | Recomb. Protein               |
| MAP                      | LSPF31-P        | LEESQVNDIDIFNSLVKSVQQEQQHNV, P. falciparum Liver-Stage Antigen 3-NRII, LSA3-NRII (81-106) peptide   | Peptides pure                 |
|                          | LSPF32-P        | DELFNELLNSVDVNGENILEESQ, P. falciparum Liver-Stage Antigen 3-NRI peptide  | Peptides pure                 |
| Malaria Parasite         | MAPF15-P        | DABCYL-ERNleFLSFP-EDANS, Malaria Aspartyl Proteinase FRET (Fluorescence Resonance Energy Transfer) Substrate I  | Substrates                    |
|                          | MAPF15-P-5      | DABCYL-ERNleFLSFP-EDANS, Malaria Aspartyl Proteinase FRET Substrate I   | Substrates                    |
| MSP-1                    | MFV11-M         | Mouse Anti-Malaria (clone 1); reacts to P.vivax/falciparum  | Antibodies                    |
|                          | MSPF11-M        | Mouse Anti-Merozoite surface protein-1 (MSP-1; P. falciparum) IgG, aff pure #1  | Antibodies                    |
|                          | MSPF11-P        | VTHESYQELVKKLEALEDAV, MSP-1 P1, peptide of P. falciparum  | Peptides pure                 |
|                          | MSPF12-P        | GYRKPLDNKDNVGMEDYIKK, MSP-1 P2, peptide of P. falciparum  | Peptides pure                 |
|                          | MSPF131P        | KLNSLNNPHNVLQNFVFFNK, MSP-1 P3, peptide of P. falciparum  | Peptides pure                 |
|                          | MSPF15-R        | Recombinant (E. coli) merozoite surface protein-1 (MSP-1; P. falciparum)  | Recomb. Protein               |
|                          | MSPF25-R        | Recombinant (E. coli) merozoite surface protein-2 (MSP-2; P. falciparum)  | Recomb. Protein               |
|                          | MSPV11-P        | LEYLREKAKMAGTLIIPES, P. vivax PvMSP-1 peptide 19 (378-397)  | Peptides pure                 |
|                          | MSPV12-P        | SKDQIKKLTSLKNLERRQN, P. vivax PvMSP-1 peptide 53 (1058-1077)  | Peptides pure                 |
|                          | MSPV13-P        | NFVGKFLLEQIPGHTDLLHL, P. vivax PvMSP-1 peptide 4 (78-97)  | Peptides pure                 |
|                          | MSPV14-M        | Mouse Anti-Merozoite surface protein-1 (MSP-1; P. vivax) IgG, aff pure #1   | Antibodies                    |
|                          | MSPV14-P        | FNQLMHVINFHYDLLRANVH, P. vivax PvMSP-1 peptide 6 (118-137)  | Peptides pure                 |
|                          | MSPV15-M        | Mouse Anti-Merozoite surface protein-1 (MSP-1; P. vivax) IgG, aff pure #2   | Antibodies                    |
|                          | MSPV15-P        | LDMLKKVVLGLWKPLDNKD, P. vivax PvMSP-1 peptide 8 (158-177)   | Peptides pure                 |
|                          | MSPV16-R        | Recombinant (E. coli) merozoite surface protein-1 (MSP-1; 108-aa; P. vivax)   | Recomb. Protein               |
|                          | MSPV26-R        | Recombinant (E. coli) merozoite surface protein-2 (MSP-2; 460-aa; P. vivax)   | Recomb. Protein               |
| (NANP)n peptides         | NANP101-P       | (NANP)10 (40-aa NANP repeat-sequence peptide of the P. falciparum CSP   | Peptides pure                 |
|                          | NANP51-A        | Anti-(NANP)5 peptide (CSP repeat, P. falciparum) IgG, aff pure  | Antibodies                    |
|                          | NANP51-BSA      | (NANP)5 peptide (CSP repeat, P. falciparum) conjugated with BSA   | Conj. Peptides                |
|                          | NANP51-P        | (NANP)5 peptide control/blocking peptide  | Peptides pure                 |
| (NVDP)n Peptides         | NVDP41-A        | Anti-(NVDP)4 peptide (minor CSP repeat-sequence P. falciparum IgG, aff pure   | Antibodies                    |
|                          | NVDP41-BSA      | (NVDP)4 peptide (CSP repeat- P. falciparum conjugated with BSA  | Conj. Peptides                |
|                          | NVDP41-P        | (NVDP)4 peptide (CSP repeat-sequence P. falciparum control/blocking peptide   | Peptides pure                 |
| (PAPP)n Peptides         | PAPP311-P       | (PAPPNAAND)3 peptide (repeat-sequence peptide of the P. berghei circumsporozoite protein, CSP), pure  | Peptides pure                 |
| RESAF15-R                | PLDH11-M        | Mouse Anti-parasite specific lactate dehydrogenase (pLDH), (PAN PLDH) IgG   | Antibodies                    |
|                          | PLDH14-M        | Mouse Anti-parasite pLDH, (P. ovale specific) IgG   | Antibodies                    |
|                          | PLDH22-M        | Mouse Anti-pLDH (P. falciparum specific) IgG  | Antibodies                    |
|                          | PLDH31-M        | Mouse Anti- pLDH (P. vivax specific) IgG  | Antibodies                    |
| (PPPNAAND)n              | PPPP312-P       | (PPPPNPPND)3 peptide (repeat-sequence of P. berghei CSP   | Peptides pure                 |
|                          | PPPP321-A       | Anti-(PPPPNAAND)3 peptide (repeat-sequence P. berghei CSP) IgG, aff pure  | Antibodies                    |
|                          | PPPP321-BSA     | (PPPPNAAND)3 peptide (repeat-sequence P. berghei CSP) conjugated with BSA   | Conj. peptides                |
|                          | PPPP321-P       | (PPPPNAAND)3 peptide (repeat-sequence P. berghei CSP) blocking peptide  | Peptides pure                 |
| HSP                      | RESAF15-R       | Recombinant Ring-infected erythrocyte surface antigen (RESA) (P.falciparum)   | Recomb. Protein               |
| Sag                      | RP-649          | Recombinant Malaria Protein Heat Shock protein (HSP)  | Pure protein                  |
|                          | SAGF11-M        | Mouse Anti-S Antigen (Sag) (P. falciparum) IgG, aff pure #1   | Antibodies                    |
| SERA                     | SAGF12-M        | Mouse Anti-S Antigen (Sag) (P. falciparum) IgG, aff pure #2   | Antibodies                    |
|                          | SERA15-R        | Recombinant (E. coli) Serine-repeat antigen (SERA) P.falciparum   | Recomb. Protein               |
| MSP                      | SP-88357-1      | MSP-1 (20 - 39), Merozoite Surface Peptide 1 (AA:Val-Thr-His-Glu-Ser-Tyr-Gln-Glu-Leu-Val-Lys-Lys-Leu-Glu-Ala-Leu-Glu-Asp-Ala-Val) (MW: 2301.60)             | Pure Peptide                  |
|                          | SP-88358-1      | MSP-1 P2, Malaria Merozoite Surface Peptide - 1 (AA: Gly-Tyr-Arg-Lys-Pro-Leu-Asp-Asn-Ile-Lys-Asp-Asn-Val-Gly-Lys-Met-Glu-Asp-Tyr-Ile-Lys-Lys) (MW: 2625.07) | Pure Peptide                  |

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