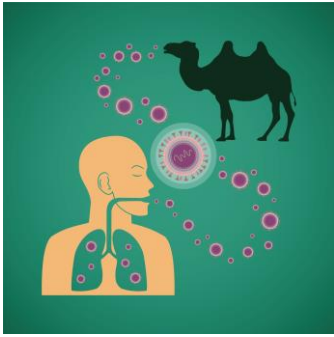


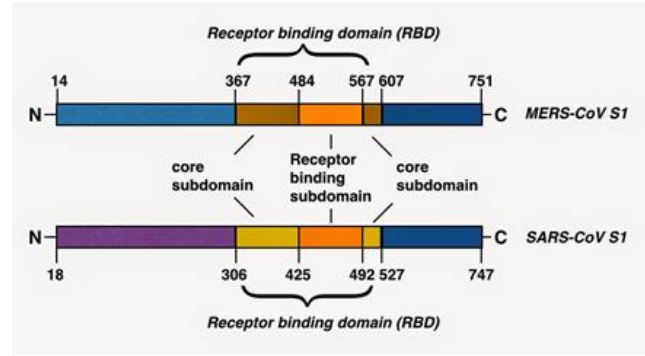
Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Vaccine and ELISA kits



MERS is a viral respiratory infection caused by the newly identified MERS-coronavirus (**MERS-CoV**). MERS-CoV is a **betacoronavirus** derived from bats. Camels have been shown to have antibodies to MERS-CoV, but the exact source of infection in camels has not been identified. Early reports compared the virus to severe acute respiratory

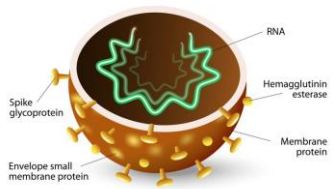
syndrome (SARS), and it has been referred to as **Saudi Arabia's SARS-like virus**. MERS can range from asymptomatic disease to severe pneumonia leading to the acute respiratory distress syndrome. MERS have high fatality rate, 77 deaths in 187 confirmed cases. MERS-CoV cases have been reported in several countries, including Saudi Arabia and the United States. Early research suggested the virus is related to one found in the bats and in dromedary camels, as 90-100% camels have antibodies to the MERS-CoV spike protein. Sera samples from European sheep, goats, cattle, and other camelids had no such antibodies. Human or animal **diagnostic** is based upon PCR or ELISA or antibody neutralization tests. **The presence of MERS viral antibodies (N, E and S) have been used to detect the infected animal or humans.**

respectively. During viral entry, the S protein is cleaved into both subunits by host cell derived proteases.



Unlike SARS-CoV, which uses human angiotensin-converting enzyme 2 (ACE2) as its receptor for binding to ACE2-expressing cells, MERS-CoV utilizes a different receptor, dipeptidyl peptidase 4 (**DPP4**), for binding to DPP4-expressing cells via the Spike protein. S1 subunit mediates virus binding to cells expressing DPP4 through its receptor-binding domain (RBD, 367-606 aa) region and an S2 subunit that mediates virus-cell membrane fusion. A truncated RBD domain (377-588)-Fc protein binds efficiently to DPP4.

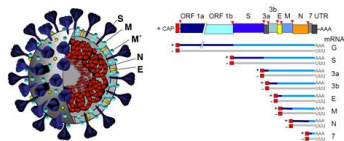
CORONAVIRUS



The virus MERS-CoV is a new member of the **beta group of coronavirus**, Betacoronavirus, **lineage C**. MERS-CoV genomes are phylogenetically classified into two clades, clade A and B. The earliest cases of MERS were of clade A clusters (EMC/2012 and Jordan-N3/2012), and new

cases are genetically distinct (clade B). MERS-CoV is distinct from SARS and distinct from the common-cold coronavirus and known endemic human betacoronaviruses HCoV-OC43 and HCoV-HKU1. MERS-CoV is more closely related to the bat coronaviruses HKU4 and HKU5 (lineage 2C) than it is to SARS-CoV (lineage 2B), sharing more than 90% sequence identity with their closest relationships, bat coronaviruses HKU4 and

CORONAVIRUS STRUCTURE AND GENE EXPRESSION



HKU5.

Coronaviruses are a positive ssRNA genome of about 27-32kb that codes for structural protein genes - namely the **Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) genes** and the Polymerase. Spike (S) protein is assembled into trimers and constitute the peplomers on the surface of the viral particle that gave the Coronaviridae its name. The S protein combines two functions, binding the host receptor and membrane fusion, which are required for viral entry into the host cell. In the case of MERS-CoV, the former is attributed to the **S1 subunit** (AA1-751) and the latter to the **S2 subunit** (AA 752-1353)

Serologic analysis of MERS-CoV: Due to the conservation of MERS viral proteins (S, N, E, M) among various coronaviruses (MERS, SARS etc) and infection of the same host and the broad distribution of CoVs in diverse mammalian species. Antibodies directed against some of the major antigens of different CoVs are known to cross-react in standard serologic assays. Potential cross-reactivity is a diagnostic challenge because camelids are known to be infected with bovine CoV (BCoV), a distinct betacoronavirus of phylogenetic lineage A unrelated to the MERS-CoV.

MERS Vaccine and Therapeutics: There are **no approved vaccine** for MERS. Inovio Pharma recently tested DNA synthetic vaccine that targets multiple MERS antigens including MERS. Novavax is testing killed virus vaccine. Nanoviricides is developing drugs that bind to viruses with virus-binding ligands in an effort to dismantle them. Antibodies to the RBD domain also protected animals from MERS infection. Therefore, MERS-CoV S1 region or the RBD are potential vaccine candidates. A **nasal** formulation of experimental vaccine has also shown promise. **Humanized antibodies to MERS** have shown success in animals. One of three monoclonal antibodies identified, m336, neutralized live and pseudotyped MERS-CoV with an exceptional potency of ID50 (half maximal inhibitory concentration) of 0.005 (pseudotyped MERS-CoV) and 0.07 (live MERS-CoV) µg/ml, respectively, by competing with the hDPP4 receptor.

About ADI's MERS ELISA Kits-ADI has cloned and expressed various MERS Rec. proteins and made antibodies. ADI has prepared antibody ELISA kits for whole spike protein (S1+S2), S1 domain, and the RBD-domain of S1, and the Nucleoprotein. Our preliminary data suggest that anti-S1-RBD and anti-NP antibody ELISA kits may provide the most specific analyses of MERS-Cov infection in humans and animals.

List of MERS ELISA Kits available from ADI.

Product details, data sheets, and pricing available (http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2795)

| Items | Kit Type | ELISA Type IgG Cat# | | | | | | | |
|-------------|----------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | | Human | Camel | Bat | Pig | Cow | Goat/sheep | Cat | Dog |
| MERS NP | Ab | RV-402100-1 | RV-402110-1 | RV-402120-1 | RV-402130-1 | RV-402140-1 | RV-402150-1 | RV-402160-1 | RV-402165-1 |
| MERS-S1 | Ab | RV-402200-1 | RV-402210-1 | RV-402220-1 | RV-402230-1 | RV-402240-1 | RV-402250-1 | RV-402260-1 | RV-402265-1 |
| MERS-S2 | Ab | RV-402300-1 | RV-402310-1 | RV-402320-1 | RV-402330-1 | RV-402340-1 | RV-402350-1 | | |
| MERS S1-RBD | Ab | RV-402400-1 | RV-402410-1 | RV-402420-1 | RV-402430-1 | RV-402440-1 | RV-402450-1 | | |

Notes: All of the ELISA kits are coated with purified Rec. proteins expressed in HEK or E. coli. There is no MERS virus or viral proteins used in the kit. So there is no risk of contamination in using these kits. All ELISA kits are for in vitro research use only (RUO), not for diagnostic procedures.

List of MERS reagents available from ADI.

| | Catalog# | Product Description | Product Type |
|----------|---------------|---|----------------|
| DPP/CD26 | DPP41-A | Rabbit anti-human (mouse, rat) DPP4 peptide IgG, aff pure | Antiserum |
| | DPP45-R-10 | Recom (HEK) Mouse Dipeptidyl peptidase 4 (DPP4) protein (29-760 a.a, hlgG1-Fc-tag, >95%, low Endotoxin) | Rec. Protein |
| | DPP46-R-10 | Recom (HEK) Human Dipeptidyl peptidase 4 (DPP4) protein (34-766 a.a, His-tag, >95%, low Endotoxin) | Rec. Protein |
| | DPP47-R-10 | Recom (HEK) Human DPP4 protein (29-766 a.a, hlgG-Fc-tag, >95%, low Endotoxin) | Rec. Protein |
| | DPP48-N-1 | Purified Human Placenta Dipeptidyl Peptidase IV (DPP4), active | Native Protein |
| MERS-RBD | MERS31-S | Rabbit Anti- Recomb (E. Coli) MERS-CoV RBD (383-502 aa) antiserum | Antibodies |
| | MERS35-R-10 | Recombinant (Sf9) MERS-CoV RBD (367-606 a.a, Rb Fc-tag, ~51 kda, >80%, low Endotoxin), active | Rec. Protein |
| | MERS36-R-10 | Recom (E. Coli) Purified MERS RBD (383-502 aa, His-tag ~15 kda) for ELISA and Western | Rec. Protein |
| | MERS37-R-10 | Recom (Sf9) Purified MERS-CoV RBD (383-502 a.a, Rb Fc-tag, ~42 kda, >85%, low Endotoxin), active | Rec. Protein |
| | MERS38-R-10 | Recom (Sf9) Purified MERS-CoV RBD (367-606 a.a, His-tag, ~28 kda, low Endotoxin), active | Rec. Protein |
| | MERS39-R-10 | Recom (Sf9) Purified MERS-CoV RBD (383-502 a.a, Mouse Fc-tag, ~44 kda, low Endotoxin), active | Rec. Protein |
| MERS-NP | MERSNP11-M | Mouse monoclonal Anti-MERS-CoV Nucleoprotein/NP (1-413 a.a) IgG, aff pure | Antiserum |
| | MERSNP12-A | Rabbit Anti-MERS-CoV Nucleoprotein/NP (1-413 a.a) IgG | Antiserum |
| | MERSNP15-R-10 | Recom (Sf9) MERS-CoV Nucleoprotein NP (1-413 aa, His-tag, ~47 kda, low Endotoxin, >95%) | Rec. Protein |
| | MERSNP16-R-10 | Recom (E. coli) MERS-CoV Nucleoprotein NP (1-413 aa, His-tag, ~47 kda, low Endotoxin, >95%) | Rec. Protein |
| MERS-S1 | MERSS126-R-10 | Recom (Sf9) Purified MERS Spike protein ECD (1-1297 a.a, His-tag, ~157 kda, low Endotoxin) | Rec. Protein |
| | MERSS12-A | Rabbit Anti-MERS-CoV Spike protein S1 protein peptide, C-terminal IgG, aff pure | Antiserum |
| | MERSS15-R-10 | Recombinant (HEK) Purified MERS-CoV Spike protein 1 (1-725 aa, His-tag, ~94 kda, low Endotoxin, >95%) | Rec. Protein |
| | MERSS16-R-10 | Recom. (Sf9) Purified MERS-CoV Spike protein S1 (18-725 a.a, His-tag, ~94 kda, low Endotoxin), active | Rec. Protein |
| | MERSS17-R-10 | Recom. (E. coli) Purified MERS-CoV Spike protein S1 (18-524 a.a, His-tag, ~62 kda) | Rec. Protein |
| | MERS121-A | Rabbit Anti-MERS Spike protein (1-1297 a.a) IgG, aff pure | Antiserum |
| | MERS122-M | Rabbit monoclonal Anti-MERS Spike protein (S1/RBD) IgG (Neutralizing) | Antibodies |
| | MERS123-M | Mouse monoclonal Anti-MERS Spike protein (S1/18-725aa) IgG (clone 1) | |
| | MERS124-M | Mouse monoclonal Anti-MERS Spike protein (S1/18-725aa) IgG (clone 2) | |
| | MERSS41-S | Anti-MERS-CoV Spike protein S1 (18-524 aa) protein antiserum | Rec. Protein |
| MERS-S2 | MERSS21-M | Mouse monoclonal Anti-MERS-CoV Spike protein S2 (726-1296 a.a) IgG, aff pure | Antiserum |
| | MERSS22-A | Rabbit Anti-MERS-CoV Spike protein S2 (726-1296 a.a) IgG, aff pure | Antiserum |
| | MERSS25-R-10 | Recom (Sf9) MERS-CoV Spike Protein S2 (726-1296 a.a, His-tag, ~66 kDa, low endotoxin) purified | Rec. Protein |

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