Plasmodium berghei, Strain (ANKA) GFP$_{CON}$ 259cl2

Catalog No. MRA-865

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Plasmodiidae*, *Plasmodium*
Species: *Plasmodium berghei*
Strain: (ANKA) GFP$_{CON}$ 259cl2
Original Source: *Plasmodium berghei (P. berghei)*, strain (ANKA) GFP$_{CON}$ 259cl2 is a genetically modified parasite derived from strain ANKA cl15cy1 following stable transfection with the pL0016 vector (BEI Resources MRA-785) containing the green fluorescent protein (GFP) gene.[1,2] Strain ANKA cl15cy1 (BEI Resources MRA-871) was cloned in Swiss mice from the 8417 (HP) clone, which was derived from the original ANKA strain.[3] *P. berghei*, strain ANKA was isolated in July 1965 from *Anopheles dureni millecampsi* mosquitoes collected in the River Kasapa, Democratic Republic of Congo.[4]
Comments: MRA-865 expresses GFP constitutively during the whole life cycle of the parasite.[1,2] MRA-865 was authenticated by the contributor and deposited to MR4 in mouse blood in 2006. The complete genome of the parent strain, *P. berghei* ANKA, has been sequenced (BioProject: PRJNA317456).[5,6]

P. berghei is a protozoan parasite that infects mammals other than humans, especially rodents, and is commonly used in rodent model studies of malaria.[6] *P. berghei* preferentially invades reticulocytes, typically producing infections in mice that induce severe pathology.[5]

The transgene from pL0016 is integrated into the genome by single cross-over integration and, therefore, parasites can lose the construct and revert to wild type; infected mice may be treated with pyrimethamine in the drinking water to prevent loss of the construct or reselection.[7] This line contains 2 to 3 copies of the construct integrated into the c-ribosomal-rna gene unit *(c-rrna)* and has a higher level of GFP expression than single *gfp* gene integrants.[1,2] Gametocyte, ookinete, oocysts, sporozoite and liver-stage development is comparable to wild type *P. berghei* (ANKA).[1]

Material Provided:
Each vial of MRA-865 contains approximately 0.5 mL of *P. berghei*-infected mouse blood in Glycerolyte 57 solution (1:2). This item is host-restricted and must be amplified in rodents. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
MRA-865 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended (-130°C or colder). Freeze-thaw cycles should be avoided.

Growth Conditions[8]:
in vivo, Swiss Webster mouse
Note: Some strains of mice may require dietary or drug pretreatment protocols for successful infection as *P. berghei* strains have a strong predilection for invasion of reticulocytes.
Inoculation:
1. Thaw a frozen cryovial of MRA-865 in a 35°C to 37°C water bath for approximately 2 to 3 minutes. Do not allow the vial to immerse near the cap line seal while thawing.
2. Once thawed, wipe the outside of the vial with 70% ethanol before opening. Using a 1 mL syringe equipped with a 27-gauge 1/2-inch needle, remove approximately 200 µL to 300 µL from the vial.
3. Wipe the injection site of the mouse with 70% ethanol and inject the sample intraperitoneally at 50 µL to 100 µL per mouse (approximately 3 mice for most applications).

Monitoring parasitemia:
1. Starting 3 days post-inoculation, monitor the growth of parasites by tail vein bleed sampling and Giemsa-stained thin blood smear microscopy at 1- to 2-day intervals.
2. Passage the strain when the infection is at or near the first peak of parasitemia (> 5%). This will normally occur within one week of inoculation.

   % parasitemia = (Infected RBC/Total RBC) × 100

Note: Do not directly inject freshly thawed parasites from cryopreserved stocks by the intravenous (IV) route, as these samples contain cryoprotectant, anticoagulant and may contain traces of lysed or coagulated red blood cells. Direct IV inoculation from cryopreserved stock may result in pulmonary embolism or shock in mice.

Passaging:
1. Anesthetize infected mice by CO_2/O_2 inhalation. Collect the blood by orbital bleeding or from the tail vein into 25 mL of cold 1× PBS-heparin anticoagulant solution (please refer to Appendix I for preparation instructions).
2. Inject the sample into each of the uninfected mice (approximately 10 mice) as described in Inoculation step #3 above.
3. Monitor parasitemia as described above and passage as needed.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium berghei*, Strain (ANKA) GFP$_{CON}$ 259cl2,

MRA-865, contributed by Chris J. Janse and Andrew P. Waters."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

All blood cultures should be handled with appropriate safety precautions necessary for the handling of bloodborne pathogens. Personnel must be trained in accordance with their institutional policy regarding bloodborne pathogens.

Disclaimers:

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References:

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8. Peters, W. and B. L. Robinson. (1999), "Chapter 92 -- Malaria." In Handbook of Animal Models of Infection. Eds. O. Zak and M. Sande, Academic Press: London, pp. 757-773.

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APPENDIX I: CRYOPRESERVATION

Note: Only immature blood-stage parasites (rings) are viable by this method. Parasitemia of 1% or higher of ring-stage parasites is recommended for cryopreservation. All steps should be carried out in a biosafety cabinet under proper air flow.

1. Prepare a 1× PBS-heparin anticoagulant solution using sterile 1× PBS (pH ~ 7.2) without calcium or magnesium (ATCC® 30-2200™) adjusted to contain 30 Units per milliliter sterile heparin.
2. Harvest parasitized mouse blood into 25 × volume ice cold sterile 1× PBS-heparin anticoagulant solution and place on ice.
3. Centrifuge the diluted blood culture at 1000 × g for 5 minutes at 4°C.
4. Aspirate the supernatant carefully. Measure the volume of packed red blood cells using centrifuge tube graduations or standard volume controls.
5. To the volume of packed red blood cells, add dropwise one volume of cold (4°C) Glycerolyte 57 solution (Fenwal, Cat. No. 4A7831, or equivalent). Let stand for 5 minutes at room temperature.
6. Add dropwise an additional volume of cold Glycerolyte 57 solution to the pellet. Mix well and aliquot 0.5 mL into 1.5 mL sterile cryopreservation vials.
7. Place the vials in a controlled-rate freezing unit. From room temperature, cool the vials at -1°C per minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per minute through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 24 to 48 hours and then plunge vials into liquid nitrogen.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).