bicarbonate solution (final =0.2% NaHCO₃), 10 ml of 200 mM L-glutamine
add 25 ml of 1 M HEPES (final = 25 mM), 27 ml 7.5% sodium
incomplete medium (final =10% serum). Store at 4
serum (MR4: type A is used with washed type O blood) to 450 ml
1.0 L. Mix thoroughly and filter with 0.22 cm sterile filter unit.  Store at
10mg/ml Gentamicin (final =2.5 ug/ml , optional).  Add TC grade water to
hypoxanthine (10 ml of 20 mM hypoxanthine stock in 1M NaOH), 0.25 ml

1. Thaw vial in 37° C water bath just until culture is completely thawed,
2. Carefully aspirate the medium with a sterile unplugged
place flask onto flask warmer in the biological safety hood.
3. To the volume of packed RBCs, add slowly dropwise one
volumes of incomplete medium; add 50 ml of appropriate heat inactivated human
4. Add an additional 3 volumes of cold Glycero1yte 57 to the
5. To make a blood film: working in the biological safety hood, asp
6. Transfer to liquid nitrogen for long term storage.

**Important notes:**
This reagent was authenticated by the contributor. Please contact malaria@atcc.org for any comment.

ALL BLOOD CULTURES SHOULD BE HANDLED WITH APPROPRIATE SAFETY PRECAUTIONS NECESSARY FOR THE HANDLING OF BLOOD BORNE PATHOGENS. PERSONNEL MUST BE TRAINED IN ACCORDANCE WITH THEIR INSTITUTIONAL POLICY REGARDING BLOOD BORNE PATHOGENS.

**Biosafety Level:** 2

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**Example of how to reference MR4 reagents:**
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