**Protocol:** Globin Reduction v1 – Removal of globin mRNA from total RNA isolated from whole blood (from GLOBINclear – Human Kit instruction manual)

**Notes:**
- do not freeze Streptavidin Magnetic Beads or RNA Binding Beads, store both at 4°C. store Capture Oligo Mix at -20°C and remaining reagents at room temperature.
- thaw frozen Capture Oligo Mix
- set constant temperature incubators to 50°C and 58°C
- the kit is optimized for globin mRNA removal from 1-10ug of input whole blood total RNA. if more than the recommended 10ug of RNA is used in the procedure, globin mRNA capture will be incomplete. the recommended maximum volume is 14ul. dry down RNA samples as needed.
- prepare reagents prior to starting procedure. add 2ml 100% isopropanol to the bottle labeled RNA Binding Buffer Concentrate. mix well and mark the label to indicate that the isopropanol was added. add 4ml 100% ethanol to the RNA Wash Solution Concentrate bottle. mix well and indicate on the label that the ethanol was added.
- prepare Bead Resuspension Mix prior to starting procedure. according to the table on page 8 of the GLOBINclear – Human Kit instruction manual, combine in a 1.5ml tube RNA Bead Buffer with RNA Binding Beads (mix the RNA Binding Beads thoroughly by vortexing before dispensing), mix briefly, and then add 100% isopropanol and mix thoroughly by vortexing.
- prepare Streptavidin Magnetic Beads prior to starting procedure. warm the 2X Hybridization Buffer and the Streptavidin Bead Buffer to 50°C for at least 15 minutes, and vortex well before use. vortex the tube of Streptavidin Magnetic Beads and aliquot 30ul for each sample to be processed. briefly centrifuge for less than 2 seconds at low speed. place the tube on a magnetic stand to capture the Streptavidin Magnetic Beads. leave the tube until the mixture becomes transparent (~5 minutes). carefully aspirate the supernatant using a pipet without disturbing the Streptavidin Magnetic Beads. discard the supernatant and remove the tube from the magnetic stand. add Streptavidin Bead Buffer to the Streptavidin Magnetic Beads; use a volume equal to the original volume of Streptavidin Magnetic Beads. vortex vigorously until beads are resuspended and keep at 50°C for at least 15 minutes before being used later in procedure.
- warm Elution Buffer to 58°C prior to using later in procedure.

1. Combine 1-10ug human whole blood total RNA (in a maximum volume of 14ul) with 1ul of Capture Oligo Mix in a 1.5ml tube. Add Nuclease-Free Water to the sample mixture as necessary to a final volume of 15ul.
2. Add 15ul of 50°C 2X Hybridization Buffer, vortex briefly to mix and centrifuge briefly for less than 2 seconds at low speed. Incubate at 50°C for 15 minutes.
3. Remove the prepared Streptavidin Magnetic Beads from the 50°C incubator and resuspend them by gentle vortexing. Briefly centrifuge for less than 2 seconds at low speed. Add 30ul of prepared Streptavidin Magnetic Beads to each RNA sample, vortex to mix well, and centrifuge briefly for less than 2 seconds at low speed. Flick the tube very gently to resuspend the beads, being careful to keep the contents at the bottom of the tube. Incubate at 50°C for 30 minutes.

4. Remove sample and vortex briefly to mix, centrifuge for less than 2 seconds at low speed. Capture the Streptavidin Magnetic Beads on a magnetic stand. Leave the tube until the mixture becomes transparent (~5 minutes). Carefully draw up the supernatant, which contains the globin mRNA depleted RNA, and transfer the RNA to a new 1.5ml tube. Place RNA on ice, discard the tube with the Streptavidin Magnetic Beads.

5. Add 100ul RNA Binding Buffer to each RNA sample. Vortex the Bead Resuspension Mix to resuspend the beads thoroughly and immediately dispense 20ul to each sample. Vigorously vortex the sample for 10 seconds, briefly centrifuge for less than 2 seconds at low speed.

6. Capture the RNA Binding Beads by placing the tube on a magnetic stand. Leave the tube until the mixture becomes transparent (~5 minutes). Carefully aspirate the supernatant without disturbing the RNA Binding Beads and discard the supernatant (it is important to remove as much of the supernatant as possible). Remove the tube from the magnetic stand.

7. Add 200ul RNA Wash Solution to each sample and vortex for 10 seconds. Briefly centrifuge for less than 2 seconds at low speed. Capture the RNA Binding Beads on a magnetic stands as in the previous magnetic bead capture steps. Carefully aspirate and discard the supernatant and remove the tube from the magnetic stand. Briefly centrifuge the tube as in previous steps and place it back on the magnetic stand. Remove any liquid in the tube with a small-bore pipet tip, remove the tube from the magnetic stand, and allow the beads to air-dry for 5 minutes with the caps left open (do not air-dry the beads for more than 5 minutes).

8. Add 30ul warm Elution Buffer to each sample, and vortex vigorously for 10 seconds to thoroughly resuspend the RNA Binding Beads. Incubate at 58°C for 5 minutes. Vortex the sample vigorously for 10 seconds to thoroughly resuspend the RNA Binding Beads and centrifuge for less than 2 seconds at low speed.

9. Capture the RNA Binding Beads on a magnetic stand as in the previous magnetic bead capture steps. Be especially careful at this step to avoid disturbing the RNA Binding Beads when collecting the supernatant. The purified RNA will be in the supernatant, and transfer to a new 1.5ml tube (frequently some of the RNA Binding Beads are carried over to the eluate, tinting it brownish but not affected further application).

10. Quantitate on NanoDrop, blank instrument with Elution Buffer. Processing whole blood total RNA with the GLOBINclear Human Kit typically reduces RNA yield by 30%.