45 min Hybridization and Bead Preparation

	Hybridization
☐	Prepare 1 - 1,000 ng total RNA in 26 µl.
☐	Add 4 µl **HS** ●.
☐	Add 5 µl **PM** ●, mix until homogeneous.
☐	Denature for 5 min at 75 °C / 1,250 rpm.
☐	Incubate for 30 min at 60 °C / 1,250 rpm.
	Bead Washing
☐	Resuspend **DB** ●, transfer 75 µl to a fresh tube.
☐	Place on magnet for 2 - 5 min, discard supernatant.
☐☐	Resuspend beads in 75 µl **DS** ●, incubate 2 min on magnet, discard supernatant. Repeat once.
☐	Resuspend beads in 30 µl **DS** ●.

45 min Depletion and Purification

	Depletion
☐	Spin down hybridized sample. Add 30 µl of prepared beads. Mix by pipetting 8x, or until homogeneous.
☐	Incubate for 15 min at 60 °C / 1,250 rpm. Spin down.
☐	Place on magnet for 5 min.
☐	Transfer 60 µl supernatant to a fresh tube. **ATTENTION:** The supernatant contains the rRNA depleted RNA.
	Purification
☐	Add 24 µl **PB** ○ and 108 µl **PS** ○, mix well, incubate for 5 min at RT.
☐	Place on magnet for 5 - 10 min, discard supernatant.
☐☐	Wash the beads twice with 120 - 150 µl 80 % EtOH, 30 sec. **ATTENTION:** Use 150 µl for 1.5 ml tubes.
☐	Air dry beads for 5 - 10 min. **ATTENTION:** do not over dry the beads!
☐	Add 12 µl **EB** ○, remove from magnet, mix well, incubate 2 min at RT.
☐	Place on magnet for 2 - 5 min, transfer 10 µl of the supernatant to a fresh tube. ☞ Safe stopping point.

ATTENTION: Spin down solutions before opening tubes or plates!

45 min Hybridization and Bead Conditioning

	Hybridization
☐	Prepare 1 - 1,000 ng total RNA in 26 µl.
☐	Add 4 µl **HS** ●.
☐	Add 5 µl **PM** ●, mix until homogeneous.
☐	Denature for 5 min at 75 °C / 400 rpm.
☐	Incubate for 30 min at 60 °C / 400 rpm.
	Bead Conditioning
☐	Resuspend **DB** ●, transfer 75 µl to a fresh tube.
☐	Place on magnet for 2 - 5 min, discard supernatant.
☐☐	Resuspend beads in 75 µl **CS** ●, incubate 2 min on magnet, discard supernatant. Repeat once.
☐☐☐	Resuspend beads in 75 µl **DS** ●, place on magnet for 2 - 5 min, discard supernatant. Repeat twice.
☐	Resuspend beads in 30 µl **DS** ●.

75 min Depletion and Purification

	Depletion
☐	Spin down hybridized sample. Add 30 µl conditioned beads. Mix by pipetting 8x, or until homogeneous.
☐	Incubate for 15 min at 60 °C / 400 rpm. Spin down.
☐	Place on magnet for 5 min.
☐	Transfer 60 µl supernatant to a fresh tube. **ATTENTION:** The supernatant contains the rRNA depleted RNA.
	Purification
☐	Add 24 µl **PB** ○ and 108 µl **PS** ○, mix well, incubate for 20 min at RT.
☐	Place on magnet for 5 - 10 min, discard supernatant.
☐	Add 30 µl **EB** ○, remove from magnet, mix well, incubate 2 min at RT.
☐	Add 66 µl **PS** ○, mix well, incubate 5 min at RT.
☐	Place on magnet for 2 - 5 min, discard supernatant.
☐☐	Wash the beads twice with 120 - 150 µl 80 % EtOH, 30 sec.
☐	Air dry beads for 5 - 10 min. **ATTENTION:** do not over dry the beads!
☐	Add 12 µl **EB** ○, remove from magnet, mix well, incubate 2 min at RT.
☐	Place on magnet for 2 - 5 min, transfer 10 µl of the supernatant to a fresh tube.

ATTENTION: Spin down solutions before opening tubes or plates!

RiBO COP™ Select and Deplete