Advanta FFPE RNA Extraction Kit

IMPORTANT Before using the reagent kit, read and understand the detailed instructions and safety guidelines in the Advanta™ FFPE RNA Extraction Kit Protocol (PN 101-6554).

Extract RNA

IMPORTANT
- Centrifuge the enzymes before use.
- Vortex and centrifuge all other buffers and reagents before use.

☐ **1** Prepare 80% ethanol fresh for each use. The total volume of 80% ethanol needed per sample is 2,000 µL (including approximately 30% overage). Scale up appropriately.

☐ **2** Add 1 mL of hexadecane to each 1.5 mL tube containing FFPE tissue samples (curls or slides, up to 2.0 mm^3 volume). Vortex the tubes at maximum speed for 10 sec, and then centrifuge for 2 sec to collect contents.

☐ **3** Incubate tubes at 55 °C for 3 min to completely melt the paraffin.

☐ **4** Centrifuge the tubes at 15,000 x *g* for 2 min at room temperature to pellet the material. Depending on the tissue input, a pellet might not be visible.

☐ **5** Remove and discard the supernatant by pipetting, removing as much as possible but being careful not to disturb the pellet.

☐ **6** Add 1 mL of 100% ethanol to each tube and mix by vortexing at maximum speed for 10 sec. Centrifuge at 15,000 x *g* for 2 min at room temperature to ensure that the contents are collected at the bottom of the tube.

☐ **7** Remove and discard the supernatant by pipetting. Do not remove any of the pellet.

☐ **8** Air-dry the pellet at room temperature for 10 min until all residual ethanol has evaporated.

☐ **9** Into each tube, add FFPE Extraction Buffer, RNA Enhancer, and Proteinase K using the volumes shown:

Component	Volume (µL)
FFPE Extraction Buffer (PN 101-6441)	100
RNA Enhancer (PN 101-6443)	4
Proteinase K (PN 101-6447)	4
Total	**108**

☐ **10** Mix by vortexing the tubes at medium speed for 5 sec and then centrifuging for 2 sec to collect contents.

☐ **11** Incubate the tubes at 55 °C for 1 hour. After incubation, vortex the tubes at medium speed for 5 sec, and centrifuge for 2 sec to collect contents. Reset the heating block temperature to 37 °C.

☐ **12** Incubate the tubes at 90 °C for 10 min.

☐ **13** While the tubes are incubating, prepare the mixture for the bead cleanup:

a Resuspend the AMPure® XP Beads by vortexing the stock bottle of beads at maximum speed for 20 sec.

b In a new set of tubes, prepare the bead mixture as shown:

Component	Volume (µL)
AMPure XP Beads (PN 101-5998)	78
FFPE Purification Reagent (PN 101-6442)	62
Total	**140**

c Label the bead mixture tubes accordingly and save them for the bead cleanup procedure.

☐ **14** After incubation in Step 12, cool the tubes to room temperature, vortex the sample tubes briefly, and centrifuge at 15,000 x *g* for 2 min at room temperature. Reset the heating block temperature to 75 °C.

Perform Bead Cleanup

☐ **1** Transfer 100 µL of the supernatant to each of the tubes containing beads that were prepared in Step 13 of the previous section. Mix well by pipetting up and down three times. Discard the tubes that contained the pellets.

☐ **2** Vortex the bead mixture tubes at medium speed for 10 sec, and then centrifuge for 2 sec to collect contents.

☐ **3** Incubate the tubes at room temperature for 10 min.

☐ **4** Place the tubes on a magnetic separator for 2 min or until the solution is clear.

☐ **5** Without disturbing the beads, and keeping the tubes on the magnetic separator, use a pipette to remove and discard the supernatant. Leave ~5 µL of the supernatant in the tube to avoid drawing out beads.

☐ **6** Wash the beads three times with 80% ethanol:

a Keeping the tubes on the separator, add 400 µL of 80% ethanol to each tube.

b Incubate the tubes at room temperature for 1 min.

c Without disturbing the beads, and keeping tubes on the magnetic separator, remove and discard all of the ethanol.

d Repeat Steps 6a–6c twice for a total of three washes.
☐ Wash 1 ☐ Wash 2 ☐ Wash 3

☐ **7** Remove the remaining ethanol by pipetting using a 20 µL tip. Air-dry the beads at room temperature for 10 min (or dry at 37 °C for 1 min) to allow residual ethanol to evaporate.

☐ **8** Prepare the eluate:

a Transfer the tubes to a rack and add 20 µL of Dilution Reagent to each tube. Flick or gently snap the bottom of the tube with your forefinger to mix the contents.

NOTE If the beads do not resuspend easily, pipet the solution up and down until the beads are completely resuspended.

b Vortex the tubes at medium speed for 5 sec, and then centrifuge for 2 sec to collect contents.

c Incubate the tubes at room temperature for 2 min.

d Place the tubes on a magnetic separator for 2 min or until the solution is clear.

☐ **9** Without disturbing the beads, and keeping the tubes on the magnetic separator, transfer each eluate (~17 µL) to a new 1.5 mL tube for DNase I digestion.

IMPORTANT Leave ~3 µL of eluate in the tube to avoid drawing out beads.

Perform DNase I Digestion

☐ **1** To each of the eluate tubes, add 2 µL of 10X DNase reaction buffer and 1 µL of DNase I. Vortex at medium speed for 5 sec, and then centrifuge for 2 sec to collect contents.

☐ **2** Incubate the tubes at 37 °C for 10 min. Centrifuge for 2 sec to collect contents.

☐ **3** Add 2 µL of 50 mM EDTA to each tube. Vortex each tube at medium speed for 5 sec and centrifuge for 2 sec.

☐ **4** Incubate at 75 °C for 10 min. After incubation, transfer the tubes to a rack and allow them to cool to room temperature for 2 min. Centrifuge the tubes for 2 sec to collect contents.

☐ **5** Add 9 µL of FFPE Purification Reagent to each of the tubes.

☐ **6** Vortex the stock bottle of AMPure XP Beads at maximum speed for 20 sec, and then add 22 µL of the beads to each tube. Flick or gently snap the bottom of the tubes to mix the contents, and then vortex the tubes at medium speed for 10 sec. Centrifuge the tubes for 2 sec to collect contents.

☐ **7** Incubate the tubes at room temperature for 10 min.

☐ **8** Place the tubes on a magnetic separator for 2 min or until the solution is clear. Without disturbing the beads, and keeping the tubes on the magnetic separator, use a pipette to remove and discard the supernatant. Leave ~5 µL of the supernatant in the tube to avoid drawing out beads.

☐ **9** Wash the beads three times with 80% ethanol:

a Keeping the tubes on the separator, add 100 µL of 80% ethanol to each tube to wash the beads.

b Incubate the tubes at room temperature for 1 min.

c Without disturbing the beads, and keeping the tubes on the magnetic separator, remove and discard the ethanol.

d Repeat Steps 9a–9c twice for a total of three washes.
☐ Wash 1 ☐ Wash 2 ☐ Wash 3

☐ **10** Remove the remaining ethanol by pipetting. Air-dry at room temperature for 10 min (or dry at 37 °C for 1 min) to allow residual ethanol to evaporate.

☐ **11** Prepare the eluate:

a Transfer the tubes to a rack and add 20 µL of Dilution Reagent to each tube. Flick or gently snap the bottom of the tube with your forefinger to mix the contents.

NOTE If the beads do not resuspend easily, pipet the solution up and down until the beads are completely resuspended.

b Vortex the tubes for 5 sec at medium speed and then centrifuge for 2 sec to collect contents.

c Incubate the tubes at room temperature for 2 min.

d Place the tubes on a magnetic separator for 2 min.

☐ **12** Without disturbing the beads, and keeping the tubes on the magnetic separator, transfer the eluate (~18 µL) to a new 1.5 mL tube.

IMPORTANT Leave ~2 µL of eluate in the tube to avoid drawing out beads.

☐ **13** Proceed with quantification/qualification immediately or store RNA samples at –20 °C for up to 7 days or at –80 °C for longer-term storage.

For technical support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com
Europe/Middle East/Africa/Russia +44 1223 859941 | techsupporteurope@fluidigm.com
Latin America +1 650 266 6100 | techsupportlatam@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com
China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com
All other Asian countries/India/Australia +1 650 266 6100 | techsupportasia@fluidigm.com

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