TECHNICAL BULLETIN

Obtain High-Quality Nucleic Acids from Organoid Cultures Using the EasySep™ Total Nucleic Acid Extraction Kit

Organoids have revolutionized disease modeling and drug discovery by providing physiologically relevant 3D structures that closely mimic human organ function. Successful nucleic acid purification underpins these models, yielding the high-quality DNA and RNA required for downstream applications such as qPCR, RNA-seq, and mutation profiling. However, because organoid sample input is often limited, sometimes to a single dome, efficient recovery is essential to ensure that molecular assays accurately represent the genome and transcriptome.

Traditional extraction methods often fall short when applied to complex 3D cultures. Phenol-chloroform-based extractions expose researchers to hazardous reagents and generate toxic waste, while manual phase separation increases the risk of cross-contamination and RNA degradation. Conversely, spin-column approaches frequently result in insufficient recovery from scarce samples and require multiple centrifugation steps that extend hands-on time. The EasySep™ Total Nucleic Acid Extraction Kit addresses these challenges with a rapid, column-free, magnetic bead-based solution. By leveraging magnetic particle chemistry downstream of STEMCELL's organoid culture media, researchers can achieve a streamlined workflow with optimal purity and ample nucleic acid recovery.

This technical bulletin provides validated protocols for epithelial and large, matrix-free organoids. A key advantage of these workflows is the ability to lyse organoids directly in the culture well immediately after media aspiration, eliminating the need for tedious matrix removal or organoid dissociation. This not only saves significant time but also reduces plasticware consumption. Depending on downstream requirements, users can choose between the Organoid Standard Protocol (for total nucleic acids with an optional RNase step) or the Organoid RNA Protocol, which features integrated DNase I digestion to ensure gDNA-free samples for sensitive applications like RT-qPCR and RNA-seq.

Materials

- EasySep™ Total Nucleic Acid Extraction Kit (Catalog #100-1079)
- ErythroClear™ Magnet (Catalog #01737)
- Costar® Microcentrifuge Tubes, 1.7 mL
- D-PBS (Without Ca++ and Mg++) (Catalog #37350)
- Ethanol (96-100%)*
- Isopropanol (100%)
- Nuclease-Free Water (Catalog #79002) OR Tris-EDTA Buffer (IDT Catalog #11-05-01-09)
- For optional RNase A treatment during Organoid Standard Protocol: RNase A, DNase and protease-free (10 mg/mL; Thermo Fisher™ Catalog #EN0531)
- For DNase I treatment during Organoid RNA Protocol: DNase I, RNase-free (1 U/µL) (Thermo Fisher™ Catalog #EN0521)

**Do not use denatured alcohol, which may contain other substances such as methanol or methylethylketone.*

Protocol: Extract Nucleic Acids from Intestinal, Hepatic, or Pancreatic Organoid Cultures

I. Organoid Standard Protocol: Total Nucleic Acid Extraction

1. Check that the domes to be harvested are intact.

   Note: *This protocol is for extraction from 1 - 2 Matrigel® (Corning 356231) domes (30 - 50 µL each) cultured in 24-well plates.*

2. Aspirate media from each well.

3. Add 200 µL of lysis buffer along with 20 µL of Proteinase K to each well.

4. Add 100 - 170 µL of PBS to each well

   Note: *The volume of PBS added is dependent on the total volume of Matrigel® going into the extraction. Add 200 µL PBS minus the total volume of Matrigel® across the 1 or 2 domes being processed.*

5. Gently triturate the mixture to homogenize the dome(s) (avoid excessive foaming).

   Note: *To process multiple domes into a single extraction, transfer the lysate mixture to the second well and continue gently triturating.*

6. Transfer lysate mixture to a 1.7 mL microcentrifuge tube and incubate at 56°C for 10 minutes.

For optional RNase treatment, proceed with steps 7 - 9, otherwise skip to step 10.

Note: *Removal of RNA from the sample may be required for some downstream applications.*

7. Allow the lysate to cool at room temperature for 2 minutes.

8. Add 80 µL of RNase A, DNase and protease-free (10 mg/mL; Thermo Fisher Catalog #EN0531) to the lysate.

9. Mix and incubate at room temperature for 5 minutes.

10. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

    Note: *Particles should appear evenly dispersed.*

11. Add 300 µL of diluted RapidSpheres™ to the sample.

12. Mix by pipetting up and down 15 times and incubate at room temperature for 5 minutes.

13. Place the sample into the ErythroClear™ Magnet and incubate at room temperature for 2 minutes.

14. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

15. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

16. Incubate at room temperature for 1 minute.

17. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

18. Repeat steps 15 - 17 two more times for a total of 3 x 1-minute washes.

19. Allow residual ethanol to evaporate at room temperature for 2 minutes. Do not remove the sample from the magnet.

    Note: *Residual ethanol can be aspirated after the first minute.*

20. Remove the sample(s) from the magnet and add 20 - 100 µL of elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.

    Note: *To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased by 10 µL. Transfer the original amount only into the final tube (step 23). Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.*

21. Mix by gently pipetting up and down to fully resuspend the pellet. Incubate at room temperature for 5 minutes.

    Note: *Avoid foaming the sample.*

22. Place the tube into the magnet and incubate at room temperature for 2 minutes.

23. Transfer the supernatant into a new tube. Extracted nucleic acids are ready for use.

II. Organoid RNA Protocol: RNA Extraction Only

1. Check that the domes to be harvested are intact.

   Note: *This protocol is for extraction from 1 - 2 Matrigel® (Corning 356231) domes (30 - 50 µL each) cultured in 24-well plates.*

2. Aspirate media from each well.

3. Add 200 µL of lysis buffer along with 20 µL of Proteinase K to each well.

4. Add 100 - 170 µL of PBS to each well.

   Note: *The volume of PBS added is dependent on the total volume of Matrigel® going into the extraction. Add 200 µL PBS minus the total volume of Matrigel® across the 1 or 2 domes being processed.*

5. Gently triturate the mixture to homogenize the dome(s) (avoid excessive foaming).

   Note: *To process multiple domes into a single extraction, transfer the lysate mixture to the second well and continue gently triturating.*

6. Transfer lysate mixture to a 1.7 mL microcentrifuge tube and incubate at 56°C for 10 minutes.

7. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

   Note: *Particles should appear evenly dispersed.*

8. Add 300 µL of diluted RapidSpheres™ to the sample.

9. Mix by pipetting up and down 15 times and incubate at room temperature for 5 minutes.

10. Place the sample into the ErythroClear™ Magnet and incubate at room temperature for 2 minutes.

11. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

12. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

13. Incubate at room temperature for 1 minute.

14. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

15. Remove the tube from the magnet and resuspend the particle pellet in 100 µL of DNase I solution (see PIS: preparation section C; 80 µL RNase-free H_2O, 10 µL 10X DNase I buffer, 10 µL DNase I (10 U/µL)).

    Note: *If particle pellet is difficult to resuspend, use a wider bore tip (ie. P1000). Avoid foaming the mixture.*

16. Incubate at room temperature for 15 minutes.

    Note: *Particles may sink to the bottom of the tube during incubation; this is expected.*

17. Place the tube into the magnet and incubate at room temperature for 2 minutes.

18. Carefully pipette* (do not pour) the supernatant (100 µL) into a new tube.

19. Add 100 µL of lysis buffer to the new tube containing supernatant.

20. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

    Note: *Particles should appear evenly dispersed.*

21. Add 150 µL of diluted RapidSpheres™ to the sample.

22. Mix and incubate at room temperature for 5 minutes.

23. Place the tube into the magnet and incubate at room temperature for 2 minutes.

24. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

25. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

26. Incubate at room temperature for 1 minute.

27. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

28. Repeat steps 25 - 27 two more times for a total of 3 x 1-minute washes.

29. Allow residual ethanol to evaporate at room temperature for 2 minutes. Do not remove the sample from the magnet.

    Note: *Residual ethanol can be aspirated after the first minute.*

30. Remove the sample(s) from the magnet and add 20 - 100 µL of elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.

    Note: *To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased by 10 µL. Transfer the original amount only into the final tube (step 33). Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.*

31. Mix by gently pipetting up and down to fully resuspend the pellet. Incubate at room temperature for 5 minutes.

    Note: *Avoid foaming the sample.*

32. Place the tube into the magnet and incubate at room temperature for 2 minutes.

33. Transfer the supernatant into a new tube. Extracted nucleic acids are ready for use.

RT = room temperature (15 - 25°C)
**Collect the entire supernatant, all at once, into a single pipette.*

Protocol: Extract Nucleic Acids from Large, Matrix-Free Organoids

I. Organoid (2X) Standard Protocol: Total Nucleic Acid Extraction

1. Transfer 1 large, matrix-free organoid* to a 1.7 mL microcentrifuge tube (e.g. Catalog #38089) using a wide bore tip.

   Note: *It is not recommended to process >1 large neural organoid in a single extraction.*

2. Aspirate excess media from the tube.

3. Add 400 µL of lysis buffer along with 40 µL of Proteinase K to the sample.

4. Add 400 µL of PBS to the sample.

5. Gently triturate the mixture to homogenize the organoid (avoid excessive foaming).

   Note: *Larger organoids may be difficult to homogenize. Use a wide bore tip if necessary. Triturate until the solution is homogenous.*

6. Transfer the lysate mixture to a new tube and incubate at 56°C for 10 minutes.

For optional RNase treatment, proceed with steps 7 - 9, otherwise skip to step 10.

Note: *Removal of RNA from the sample may be required for some downstream applications.*

7. Allow the lysate to cool at room temperature for 2 minutes.

8. Add 160 µL of RNase A, DNase and protease-free (10 mg/mL; Thermo Fisher Catalog #EN0531) to the lysate.

9. Mix and incubate at room temperature for 5 minutes.

10. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

    Note: *Particles should appear evenly dispersed.*

11. Add 600 µL of diluted RapidSpheres™ to the sample.

12. Mix by pipetting up and down 15 times and incubate at room temperature for 5 minutes.

13. Place the sample into the ErythroClear™ Magnet and incubate at room temperature for 2 minutes.

14. Carefully pipette* out the supernatant. Do not remove the sample(s) from the magnet. Discard supernatant.

15. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

16. Incubate at room temperature for 1 minute.

17. Carefully pipette* out the supernatant. Do not remove sample(s) from the magnet. Discard supernatant.

18. Repeat steps 15 - 17 two more times for a total of 3 x 1-minute washes.

19. Allow residual ethanol to evaporate at room temperature for 2 minutes. Do not remove the sample from the magnet.

    Note: *Residual ethanol can be aspirated after the first minute.*

20. Remove the sample(s) from the magnet and add 40 - 200 µL of elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.

    Note: *To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased by 10 µL. Transfer the original amount only into the final tube (step 23). Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.*

21. Mix by gently pipetting up and down to fully resuspend the pellet. Incubate at room temperature for 5 minutes.

    Note: *Avoid foaming the sample.*

22. Place the tube into the magnet and incubate at room temperature for 2 minutes.

23. Transfer the supernatant into a new tube. Extracted nucleic acids are ready for use.

II. Organoid (2X) RNA Protocol: RNA Extraction Only

1. Transfer 1 large, matrix-free organoid* to a 1.7 mL microcentrifuge tube (e.g. Catalog #38089) using a wide bore tip.

   Note: *It is not recommended to process >1 large neural organoid in a single extraction.*

2. Aspirate excess media from the tube.

3. Add 400 µL of lysis buffer along with 40 µL of Proteinase K to the sample.

4. Add 400 µL of PBS to the sample.

5. Gently triturate the mixture to homogenize the organoid (avoid excessive foaming).

   Note: *Larger organoids may be difficult to homogenize. Use a wide bore tip if necessary. Triturate until the solution is homogenous.*

6. Transfer lysate mixture to a new tube and incubate at 56°C for 10 minutes.

7. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

   NOTE: *Particles should appear evenly dispersed.*

8. Add 600 µL of diluted RapidSpheres™ to sample.

9. Mix by pipetting up and down 15 times and incubate at room temperature for 5 minutes.

10. Place the sample into the ErythroClear™ Magnet and incubate at room temperature for 2 minutes.

11. Carefully pipette* out the supernatant. Do not remove the sample(s) from the magnet. Discard supernatant.

12. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

13. Incubate at room temperature for 1 minute.

14. Carefully pipette* out the supernatant. Do not remove the sample(s) from the magnet. Discard supernatant.

15. Remove the tube from the magnet and resuspend the particle pellet in 200 µL of DNase I solution (see PIS: preparation section C; 160 µL RNase-free H_2O, 20 µL 10X DNaseI buffer, 20 µL DNase I (10 U/µL)).

    Note: *If particle pellet is difficult to resuspend, use a wider bore tip (ie. P1000). Avoid foaming the mixture.*

16. Incubate at room temperature for 15 minutes.

    Note: *Particles may sink to the bottom of the tube during incubation; this is expected.*

17. Place the tube into the magnet and incubate at room temperature for 2 minutes.

18. Carefully pipette* (do not pour) 200 µL of supernatant into a new tube.

19. Add 200 µL of lysis buffer to the new tube containing supernatant.

20. Shake the bottle of diluted RapidSpheres™ (see PIS: preparation section A) for 20 seconds.

    Note: *Particles should appear evenly dispersed.*

21. Add 300 µL of diluted RapidSpheres™ to the sample.

22. Mix and incubate at room temperature for 5 minutes.

23. Place the tube into the magnet and incubate at room temperature for 2 minutes.

24. Carefully pipette* out the supernatant. Do not remove the sample(s) from the magnet. Discard supernatant.

25. Add 1 mL of 70% ethanol wash solution (see PIS: preparation section B). Avoid disturbing the magnetic pellet.

26. Incubate at room temperature for 1 minute.

27. Carefully pipette* out the supernatant. Do not remove the sample(s) from the magnet. Discard supernatant.

28. Repeat steps 25 - 27 two more times for a total of 3 x 1-minute washes.

29. Allow residual ethanol to evaporate at room temperature for 2 minutes. Do not remove the sample from the magnet.

    Note: *Residual ethanol can be aspirated after the first minute.*

30. Remove the sample(s) from the magnet and add 40 - 200 µL of elution buffer (i.e. Nuclease-Free Water or Tris-EDTA buffer) directly to the pellet.

    Note: *To avoid carryover of RapidSpheres™ into the final sample, this volume may be increased by 10 µL. Transfer the original amount only into the final tube (step 33). Use a lower elution volume if a higher concentration is required. Resuspension in smaller volumes may require additional pipetting.*

31. Mix by gently pipetting up and down to fully resuspend the pellet. Incubate at room temperature for 5 minutes.

    Note: *Avoid foaming the sample.*

32. Place the tube into the magnet and incubate at room temperature for 2 minutes.

33. Transfer the supernatant into a new tube. Extracted nucleic acids are ready for use.

RT = room temperature (15 - 25°C)

**Collect the entire supernatant, all at once, into a single pipette.*

Supporting Data: Obtain High-Integrity RNA from Epithelial Organoids

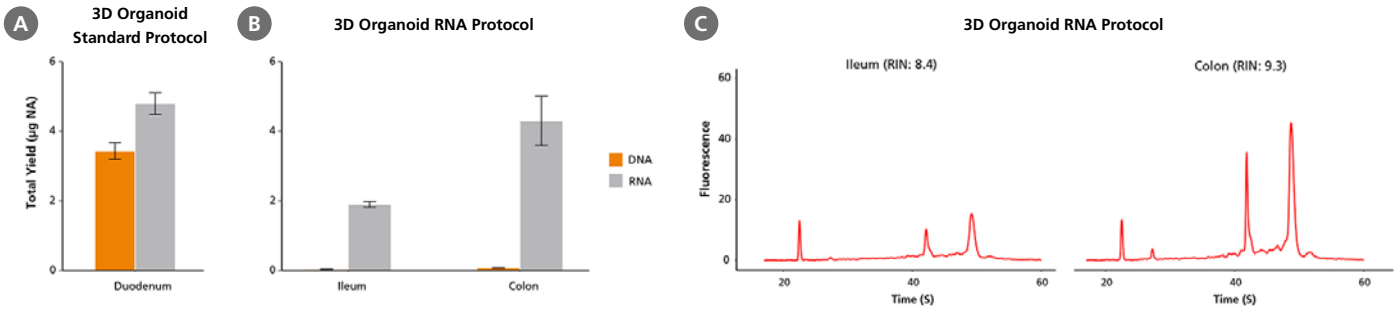

Figure 1. Efficient Nucleic Acid Recovery from Intestinal Organoid Cultures Can Be Achieved with the EasySep™ Total Nucleic Acid Extraction Kit Organoid Protocols

(A) Recovery of DNA and RNA using the Organoid Standard Protocol from intestinal organoids initiated from duodenum fragments cultured in IntestiCult™ Organoid Growth Medium (Human). 1 x 30 µL Matrigel® dome was used per extraction, with two extraction replicates per condition. Error bars represent SD. (B) Recovery of RNA and depletion of gDNA using the Organoid RNA Protocol on intestinal organoids initiated from ileum and colon fragments cultured in IntestiCult™ Plus Organoid Growth Medium (Human). 1 x 30 µL Matrigel® dome was used per extraction, with two extraction replicates per condition. Error bars represent SD. Concentrations were measured using the Qubit™ Fluorometer (dsDNA Broad Range and RNA Broad Range kits). (C) Agilent Bioanalyzer RNA 6000 Nano pherogram showing optimal RNA integrity number (RIN) scores from samples extracted using the Organoid RNA Protocol.

Table 1. Summary of Nanodrop™ Spectrophotometer Readings from Intestinal Organoid Extractions

Tissue Source	Culture Medium	Isolation Protocol	260/230	260/280
Colon	IntestiCult™ Plus OGM (Human)	Organoid RNA Protocol	2.13 ± 0.04	2.06 ± 0.02
Ileum	IntestiCult™ Plus OGM (Human)	Organoid RNA Protocol	2.13 ± 0.01	2.03 ± 0.01
Duodenum	IntestiCult™ OGM (Human)	Organoid Standard Protocol	1.99 ± 0.06	2.00 ± 0.00

OGM = Organoid Growth Medium

Supporting Data: Obtain High-Integrity RNA from Epithelial Organoids

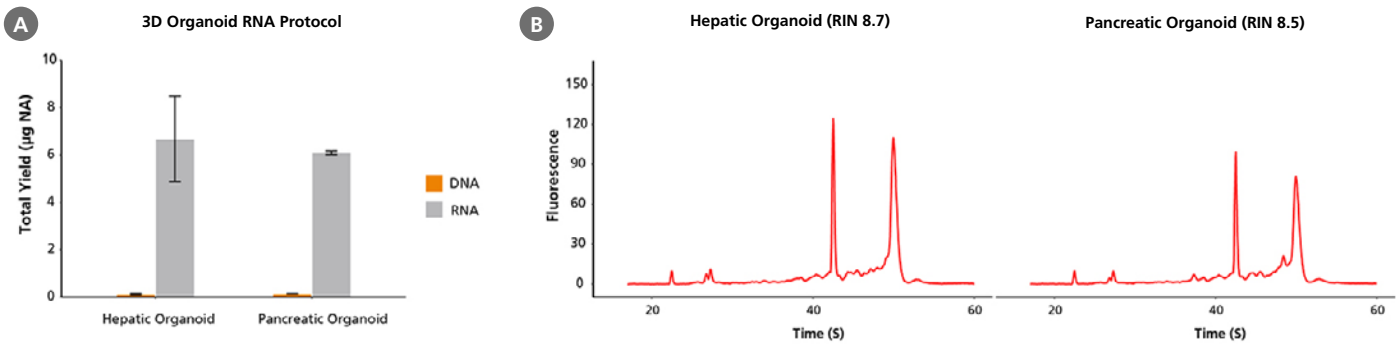

Figure 2. Isolation of High-Integrity RNA with Minimal gDNA Contamination Can Be Achieved Using the EasySep™ Total Nucleic Acid Extraction Kit Organoid RNA Protocol

(A) Normalized recovery of RNA per 30 µL Matrigel® dome across two organoid lines. Human pancreatic ductal organoids cultured in PancreaCult™ Organoid Media (Human) and hepatic organoids cultured in HepatiCult™ Organoid Growth Medium (Human). Two extraction replicates were used per condition. Error bars represent SD. Concentrations were measured using the Qubit™ Fluorometer (dsDNA Broad Range and RNA Broad Range kits). (B) Agilent Bioanalyzer RNA 6000 Nano pherogram showing optimal RNA integrity number (RIN) scores from samples extracted using the Organoid RNA Protocol.

Supporting Data: Obtain High-Quality Nucleic Acids from Large, Matrix-Free Organoids

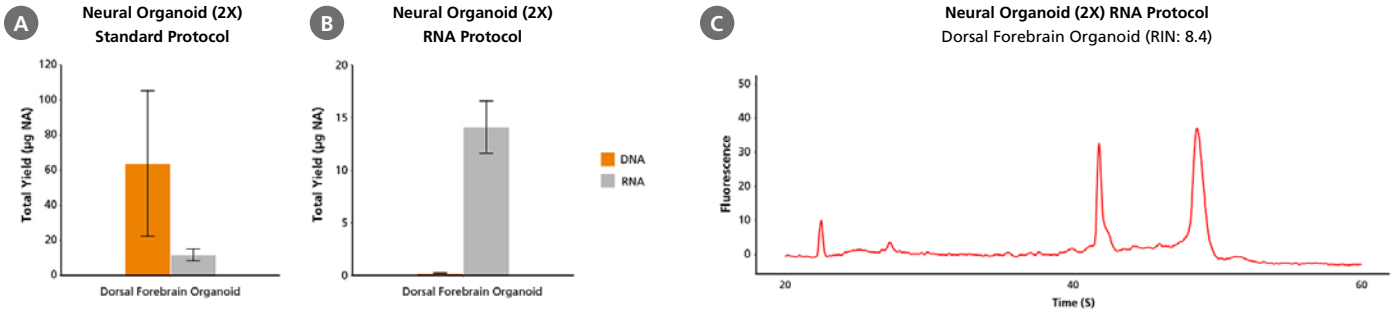

Figure 3. Efficient Nucleic Acid Recovery from Dorsal Forebrain Organoid Cultures Can Be Achieved with the EasySep™ Total Nucleic Acid Extraction Kit Large, Matrix-Free Organoid (2X) Protocols

(A) Recovery of DNA and RNA using the Large, Matrix-Free Organoid (2X) Standard Protocol from one Day-96 hPSC-derived dorsal forebrain organoid (derived using the STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit) per extraction, across 2 extraction replicates. Error bars represent SD. Wide variation in results was seen as a result of variation in input organoid size. (B) Recovery of RNA and depletion of gDNA using the Large, Matrix-Free Organoid (2X) RNA Protocol from one dorsal forebrain organoid per extraction (culture conditions same as A), across 2 extraction replicates. Error bars represent SD. Concentrations were measured using the Qubit™ Fluorometer (dsDNA Broad Range and RNA Broad Range kits). (C) Agilent Bioanalyzer RNA 6000 Nano pherogram showing optimal RNA integrity number (RIN) scores from samples extracted using the Large, Matrix-Free Organoid (2X) RNA Protocol.

Table 2. Summary of Nanodrop™ Spectrophotometer Readings from Neural Organoid Extractions

Tissue Source	Culture Medium	Isolation Protocol	260/230	260/280
Day-96 Dorsal Forebrain Organoid	STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit	Large, Matrix-Free Organoid (2X) Standard Protocol	2.05 ± 0.30	1.88 ± 0.00
		Large, Matrix-Free Organoid (2X) RNA Protocol	2.23 ± 0.00	2.04 ± 0.01