Open Access

*OBM Neurobiology*

LIDSEN Publishing Inc.

Original Research

# Breaking Barriers: A Reliable Method for RNA Extraction in Zebrafish Neurodegenerative Research

Mahintaj Dara [1, *], Zahra Karimi [2], Mehdi Dianatpour [1, 3], Hadi Aligholi [2, 4]

1. Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; E-Mails: dara.mahintaj@gmail.com; mdianatpur@gmail.com
2. Department of Neuroscience, School of Advanced Medical Science and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran; E-Mails: zahra_karimi1361@yahoo.com; aligholi@sums.ac.ir
3. Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Epilepsy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

* **Correspondence:** Mahintaj Dara; E-Mail: dara.mahintaj@gmail.com

**Academic Editor:** Francesco Amenta

*OBM Neurobiology*
2024, volume 8, issue 3
doi:10.21926/obm.neurobiol.2403246

**Received:** June 02, 2024
**Accepted:** September 18, 2024
**Published:** September 30, 2024

**Abstract**

Zebrafish (*Danio rerio*) is a vertebrate with unique characteristics, making it an excellent biomedical research animal model. One research area involves investigating changes in gene expression during neurodegenerative processes, aging-related changes, and behavioral studies in zebrafish. To achieve this, high-quality and quantity RNA must be extracted from zebrafish. This study aims to develop an optimized RNA extraction method from zebrafish larvae brains. After rearing and maintaining the zebrafish in suitable conditions, the larvae were separated, and their brains were extracted. Using an optimized TRIzol-based method, RNA was extracted from the zebrafish larvae's brains. For comparison, we also employed commercial kits and Trizol for RNA extraction. The quantity and quality of the extracted RNA were measured using NanoDrop, gel electrophoresis, and real-time PCR. We selected five genes: *gapdh* and *beta-actin* as housekeeping genes and three other genes expressed in the brain and nervous system (*bdnf, chd8, and lrp6*). According to NanoDrop results, all samples

were purified with minimal protein and polysaccharide contamination, and the 260/280 ratio and 260/230 ratio fell within the standard range of 1.9-2.2. The amount of extracted RNA significantly increased compared to other methods (P value < 0.0001), and all the studied genes exhibited high expression. RNA extraction demonstrated both high quality and quantity. In summary, we have developed an optimized method for RNA extraction from the brains of zebrafish larvae.

**Keywords**
Zebrafish; *Danio rerio*; RNA extraction; larvae

## 1. Background

The zebrafish *(Danio rerio*) is a vertebrate with unique characteristics that make it a suitable animal model system compared to other laboratory animals [1]. Its metabolic characteristics are similar to those of humans, so it is used to characterize human diseases, assess in vivo toxicity in response to treating a chemical of interest, and test new drugs to treat the diseases [2].

Zebrafish have external fertilization and high fecundity, generating many transparent embryos that develop rapidly every time [3]. These features make zebrafish an excellent model system for biomedical research and genetic manipulation [4]. In zebrafish, the central nervous system (CNS) is closely related to CNS design and connectivity in humans, which makes them an ideal choice to model the development of neurodegeneration disorders and behavioral studies [5].

One of the studied fields is investigating changes in the expression of different genes during neurodegenerative processes, aging-related changes, and behavioral studies in zebrafish [6].

For this purpose, RNA should be extracted from zebrafish. There are different methods for extracting RNA from various life cycle stages and different zebrafish tissues [7].

Because of the small size and the small organs of zebrafish, pooled samples are usually used for RNA extraction. Quality and quantity of extracted RNA are important because sample degradation or impurities can lead to false results in downstream experimental applications. In this study, we aimed to develop an optimized RNA extraction method from zebrafish larvae brains.

## 2. Methods

### *2.1 Fish Husbandry and Embryo Collection*

Adult zebrafish *(Danio rerio*) were maintained at 28 ± 1°C on a 14:10 dark-light cycle. Breeding was carried out according to international guidelines. Fertilized embryos were incubated at 28 ± 1 in embryo medium (EM: 0.137 M NaCl, 5.4 mM KCl, 0.25 mM $Na_2HPO_4$, 0.44 mM $KH_2PO_4$, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM $NaHCO_3$) [8]. Experiments were done according to the protocol approved by the Shiraz University of Medical Sciences Institutional Ethics Committee.

Experiments were done according to the protocol approved by the Shiraz University of Medical Sciences Institutional Ethics Committee. (IR.SUMS.REC.18452).

### *2.2 Larval Zebrafish Brain Extraction Process*

First, 7-day-old zebrafish larvae were anesthetized in distilled water and 0.02% MS222 (3-aminobenzoic acid ethyl ester methane sulfonate salt). Then, the larvae were fixed with insect pins in a petri dish containing artificial cerebrospinal fluid (CSF) and agarose gel. Using a needle, we removed the head's soft tissue under a stereo microscope, and once the brain tissue was exposed, we cut the spinal cord at the junction between the spinal cord and brain stem, extracted the whole brain, and contained it in an RNA later solution (YTA-Iran) kept at -80 degrees to extract RNA.

### *2.3 The RNA Extraction Procedure*

Each larval zebrafish brain was placed into 1.5-ml RNase-free microtubes and frozen at -80°C for storage. In this study, 200 zebrafish larval brains were used. At the same time, we performed RNA extraction steps from zebrafish larval brains using TRIzol solution (TRIzol™Thermo Fisher Scientific), RNX plus solution (Cinnagen-Iran), as well as commercial kit (RNeasy Kits Qiagen-Cat. No: 74004) commercial kit according to their manufactures to compare our work with existing methods. Extraction steps using Trizol, RNX plus, and Qiagen kit were performed precisely according to their protocol. Therefore, their protocol is not given here.

According to our optimized protocol, RNA extraction from zebrafish larval brain step by step of bellow was done:

1. 750 µl ice-cold RNX plus a solution was added to each micro tube.
2. Vortexed for 10-15 sec and incubated at room temperature for 5 min.
3. 200 µl of chloroform (EMSURE® ACS, ISO, Reag. Ph Eur. Merck) was added and mixed well for 15 secs by shaking.
4. The samples were incubated on ice for 5 min and centrifuged for 15 min at 12000 rpm at 4°C.
5. Then the aqueous phase was transferred to a new RNase-free 1.5 ml tube.
6. An equal volume of the liquid phase plus 50 microliters of isopropanol (Sigma-Aldrich) was added to the sample tubes, gently mixed, and incubated at -20°C for 30 min. (We used more isopropanol than usual and incubated at -20°C instead of 4°C to improve the quality and quantity of RNA extraction.)
7. Then, the mixture was centrifuged at 12000 rpm at 4°C for 15 min.
8. The supernatant was discarded gently, and 800 µl of ice-cold 70% ethanol was (Sigma-Aldrich).
9. After that, it was centrifuged at 4°C at 7500 rpm for 10 min.
10. The supernatant was discarded gently, and the pellet was set to dry at room temperature for a few minutes.
11. The pellet was dissolved in 50 µl of DEPC-treated water (Thermo Fisher Scientific). The tube was placed in a 55-60°C water bath for 5 min to dissolve the pellet better and kept at -80°C.

Using Nano drop (Nano Drop™ 2000/2000c Spectrophotometers. Thermo Fisher), the isolated RNA's purity and quantity were determined by calculating 260/280 UV absorption ratio. Isolated RNA was run on 2% agarose gel during agarose (YTA, Iran. Cat.No:YT9060) gel electrophoresis to evaluate the integrity of total RNAs.

### *2.4 Real-Time PCR*

Real-time PCR using housekeeping genes as internal controls indicate perfect nucleic acid extraction, quality of samples, and quality of PCR. Detection of some genes with Ct values within a range will indicate the samples have been collected, transported, and stored correctly. We chose five genes, *gapdh,* and *beta-actin* as housekeeping genes and three other genes expressed in the brain and nervous system (*bdnf*, *chd8*, and *lrp6*). Primer design was done using https://primer3.ut.ee/ and the specificity of the primers was checked using https://www.ncbi.nlm.nih.gov/tools/primer-blast/. (Table 1). The cDNA was synthesized with Add Script cDNA Synthesis Kit (Add-bio, Korea). For RT–PCR 1 µg of total RNA and oligo (dT) primer was used following the suppliers. RT reactions were performed at 25°C for 10 min; 60 min at 50°C and 80°C for 5 min. cDNA (1 µl) was used as a template for real-time PCR. PCR reactions were performed in a total volume of 20 µl; 10 µl RealQ Plus 2x master mix green (Ampliqon), 7 µl distilled water, 1 µl forward primer, 1 µl reverse primer, and 1 µl cDNA. Then real-time PCR was optimized to determine the number of cycles that would allow product detection within the linear phase of mRNA transcript amplification. The conditions for the reaction were as follows: Holding stage: initial 15 min denaturation step at 95°C, cycling stage: 10 s at 95°C, 1 min annealing, and extension step at 57°C, for 40 cycles.

**Table 1** Primer Sequence.

| Primer | | Sequence (5' → 3') | Size (bp) | Product size (bp) |
|---|---|---|---|---|
| *lrp6* (low-density lipoprotein receptor-related protein 6) | F | AAACTTTACTGGACCGACTCC | 21 | 90 |
| | R | CAGGTCCTGCCAGAATAACA | 20 | |
| *bdnf* (brain-derived neurotrophic factor) | F | GCACGGCAGAAGTTCTCATA | 20 | 107 |
| | R | CCTATGCTGTCCTGGAGATAGA | 22 | |
| *chd8* (Chromo domain Helicase DNA Binding Protein 8) | F | CCAGAGAGTAGGAGAGGATAGAG | 23 | 100 |
| | R | CAGTGATTAGGGAGTGAGGAATAG | 24 | |
| *gaphd* (glyceraldehyde-3-phosphate dehydrogenase) | F | GCCGTTACAAGGGAGAAGTT | 20 | 78 |
| | R | TGCACTGGAACACAGAGATG | 20 | |
| *actb* (Actin Beta) | F | AGACTCCTATGTGGGAGATGAG | 22 | 95 |
| | R | TGTCATCCCAGTTAGTCACAATAC | 24 | |

### *2.5 Statistical Analysis*

All statistical data was analyzed using and a Comparison of variables between groups was made using a One-Way ANOVA or Kruskal-Wallis H test, as appropriate. The Graph Pad Prism software V9.0 was used to analyze the data. A p-value < 0.05 was considered statistically significant.

## 3. Results

We successfully adapted a protocol to isolate RNA from the brains of zebrafish larvae. The amount of RNA extracted by this method was significantly increased (P value < 0001) compared to other methods (Figure 1A). The average concentration of RNAs was 300 ng per microliter (Table 2). The quality of extracted RNA based on the 260/280 and 260/230 ratios were in the acceptable range

of 1.8 to 2.2. The samples extracted by the optimized method had minimal protein contamination and the same quality as those extracted with the commercial kit (Figure 1B). Regarding polysaccharide contamination and the ratio of 260/230, the quality of the samples extracted with the kit was better than the other methods, and the optimized method was similar to TRIzol and RNX-plus (Figure 1C). The extracted RNA was observed on the gel with two bands of 28S rRNA and 18S rRNA separately (Figure 1D). Real-time PCR results showed that the samples extracted with the optimized method showed more expression and, as a result, lower Ct than other methods. This result was obtained for internal control genes gapdh and act-b and other genes (Figure 1E-I).

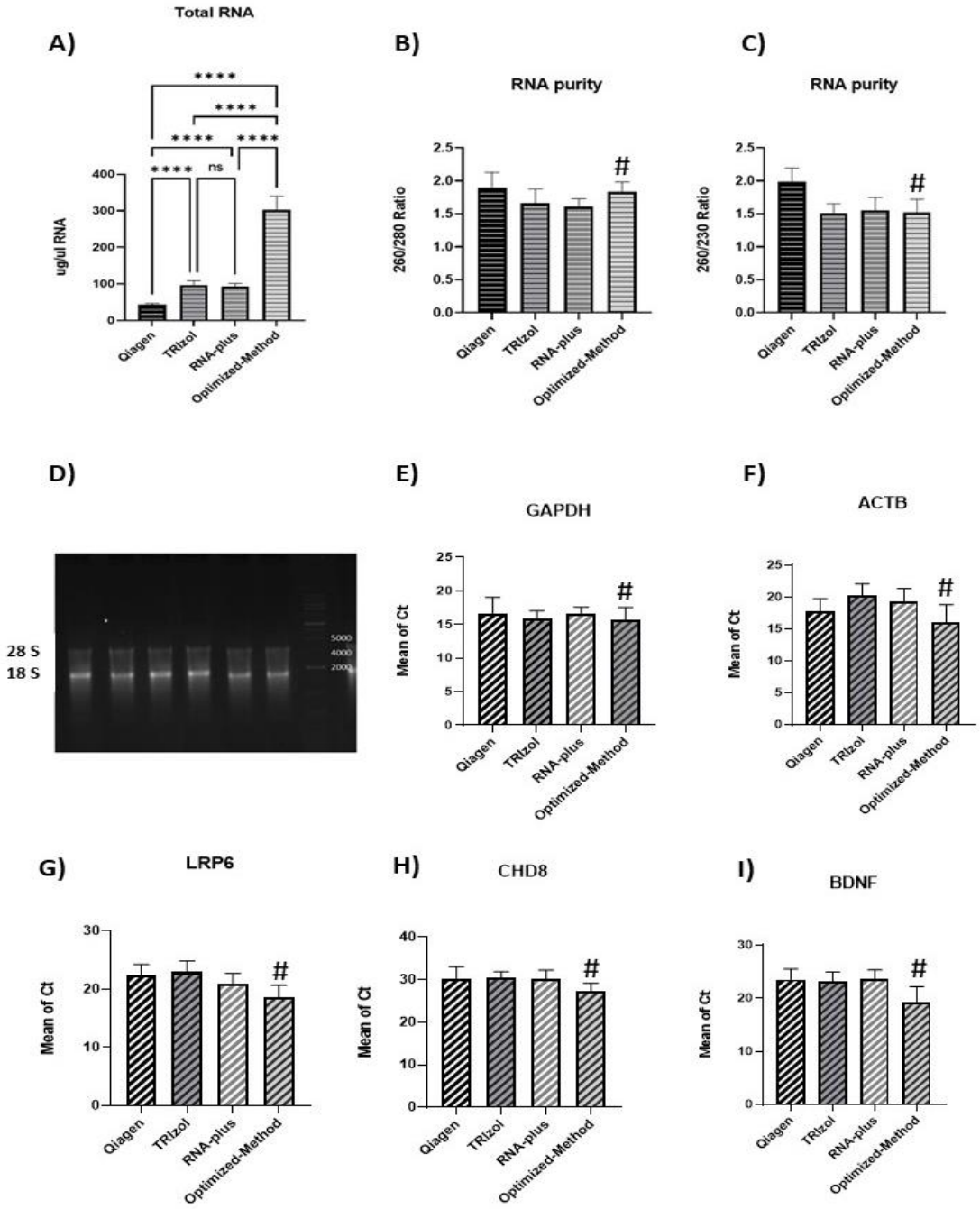


**Figure 1** A) Concentration of extracted RNA from zebrafish brain larva using optimized method in comparison with other methods. All statistical data were analyzed, and variables between groups were compared using either a One-Way ANOVA or a Kruskal-

Wallis H test, as appropriate. A p-value < 0.05 was considered statistically significant. B) RNA purity according to 260/280 ratio. The samples extracted using the optimized method had minimal protein contamination and were the same quality as those extracted with the commercial kit. C) RNA purity according to 260/230 ratio. Regarding polysaccharide contamination and the ratio of 260/230, the quality of the samples extracted with the kit was better than the other methods, and the optimized method was similar to TRIzol and RNX-plus. D) Agarose gel electrophoresis of extracted RNA using an optimized method. E) Real time PCR result for *gapdh* gene according to mean of Ct value. F) Real time PCR result for *act.B* gene according to mean of Ct value. G) Real time PCR result for *lrp6* gene according to mean of Ct value. H) Real time PCR result for *chd8* gene according to mean of Ct value. I) Real-time PCR result for *bdnf* gene according to mean of Ct value. E to I: The sample extracted using the optimized method shows a lower Ct value, indicating that the RNA expression profile is better preserved than other methods. (The # symbol is used to predict results from the optimized method compared to other methods. The * symbol is used to show statistical significancy).

**Table 2** RNA concentration from zebrafish brain larva using four different methods.

| SAMPLE ID | QIAGEN | TRIZOL | RNX-PLUS | OPTIMIZED METHOD |
|---|---|---|---|---|
| **1** | 48.9 | 104.8 | 78.3 | 348.9 |
| **2** | 42.2 | 79.5 | 79.6 | 282.2 |
| **3** | 42.3 | 99.7 | 105.5 | 336.3 |
| **4** | 39.0 | 72.9 | 102.4 | 318.1 |
| **5** | 38.3 | 92.5 | 104.3 | 265.7 |
| **6** | 46.4 | 88.3 | 93.0 | 319.4 |
| **7** | 44.8 | 94.2 | 72.6 | 267.0 |
| **8** | 47.2 | 85.0 | 102.9 | 292.3 |
| **9** | 52.9 | 92.7 | 94.7 | 312.1 |
| **10** | 45.6 | 107.1 | 92.8 | 284.5 |
| **11** | 38.7 | 90.6 | 90.9 | 303.2 |
| **12** | 44.5 | 79.1 | 80.8 | 268.2 |
| **13** | 33.5 | 105.1 | 100.2 | 324.9 |
| **14** | 40.3 | 81.2 | 94.4 | 308.1 |
| **15** | 39.6 | 92.8 | 92.7 | 323.1 |
| **16** | 39.4 | 95.6 | 100.2 | 262.4 |
| **17** | 38.5 | 112.1 | 92.4 | 333.3 |
| **18** | 40.3 | 96.6 | 93.5 | 285.7 |
| **19** | 41.9 | 77.3 | 99.0 | 293.4 |
| **20** | 46.5 | 97.6 | 95.3 | 252.6 |
| **21** | 34.0 | 106.0 | 103.7 | 281.7 |
| **22** | 42.8 | 101.1 | 78.7 | 287.0 |
| **23** | 46.3 | 90.7 | 88.1 | 229.1 |
| **24** | 51.1 | 114.5 | 94.8 | 275.4 |
| **25** | 39.7 | 96.4 | 95.2 | 276.1 |

| | | | | |
|---|---|---|---|---|
| **26** | 44.3 | 96.1 | 92.4 | 332.2 |
| **27** | 46.6 | 105.4 | 91.7 | 382.2 |
| **28** | 42.2 | 114.0 | 96.4 | 280.7 |
| **29** | 44.7 | 99.9 | 85.3 | 258.3 |
| **30** | 45.8 | 99.0 | 102.6 | 453.5 |
| **31** | 43.2 | 103.5 | 89.4 | 273.1 |
| **32** | 44.8 | 98.6 | 89.8 | 366.3 |
| **33** | 44.3 | 83.5 | 95.4 | 279.2 |
| **34** | 38.6 | 95.9 | 85.2 | 297.3 |
| **35** | 47.4 | 121.0 | 90.6 | 298.8 |
| **36** | 40.0 | 102.5 | 91.6 | 330.1 |
| **37** | 39.3 | 112.8 | 90.6 | 355.9 |
| **38** | 38.7 | 111.0 | 89.6 | 336.0 |
| **39** | 41.6 | 91.0 | 95.2 | 310.9 |
| **40** | 39.0 | 91.8 | 94.0 | 288.2 |
| **41** | 41.7 | 102.3 | 103.3 | 305.4 |
| **40** | 43.4 | 116.4 | 98.1 | 290.1 |
| **43** | 42.4 | 93.6 | 106.4 | 303.6 |
| **44** | 49.8 | 91.6 | 83.9 | 299.4 |
| **45** | 45.1 | 96.8 | 102.4 | 318.1 |
| **46** | 48.4 | 84.8 | 76.1 | 248.7 |
| **47** | 46.9 | 113.9 | 78.9 | 332.3 |
| **48** | 47.9 | 83.5 | 105.0 | 277.4 |
| **49** | 45.8 | 106.6 | 84.8 | 274.3 |
| **50** | 43.1 | 102.7 | 105.0 | 284.3 |

## 4. Discussion

Zebrafish have been the leading excellent animal model in biological research due to powerful genetic tools and standardized protocols for development [9]. However, factors like dietary requirements, lifestyle, and microbiomes are different from those in humans, but alternative animal model systems are used in parallel [8]. Because of easy genetic manipulation, a fully sequenced genome, High fertility, external fertilization, rapid growth, and producing transparent embryos, zebrafish are a unique model animal for biomedical research, including studies of human diseases and human diseases [10].

Recently, studies have shown the suitability of zebrafish as a model for studying the nervous system and related diseases such as Multiple sclerosis (MS), Parkinson's, Alzheimer's, etc., as well as complex behavioral studies such as learning and memory, aggression, anxiety, and sleep [11, 12]. Among the studies of interest are investigating gene expression changes during diseases related to the nervous system, behavioral studies, and gene expression changes during receiving drugs or special treatment compared to the control group [13, 14]. For this purpose, it is necessary to extract RNA with high quality and quantity because the quality and amount of extracted RNA can affect the results of subsequent tests such as real-time PCR. In a study, Mazzolini et al. isolated RNA from pooled adult zebrafish brains using a commercial kit. They outline a detailed protocol for isolating

specific cell types—neurons, macrophages, and microglia—from larval zebrafish brains, emphasizing the importance of maintaining gene expression profiles throughout the process. Their method avoids enzymatic digestion, which could compromise RNA quality. The resulting high-quality RNA is suitable for downstream applications such as quantitative PCR (qPCR) and transcriptomic analysis, making this protocol valuable for neuroscientific research and providing insights into the roles of these cell types in brain development and disease [15].

In another study, Xiao et al. investigate the molecular effects of the environmental pollutant 2,6-dichloro-1,4-benzoquinone (DCBQ) on adult zebrafish, focusing on changes in gene expression in the heart and brain. They used a commercial kit to extract RNA from adult zebrafish brains [16].

Shukla et al. present a refined protocol for isolating high-quality total RNA from adult zebrafish's liver, kidney, and brain tissues, which is crucial for gene expression analysis. They emphasize the necessity of intact RNA, as degraded or impure samples can result in unreliable outcomes in downstream applications such as real-time PCR. They employ a commercially available chemical denaturant and a cleanup process to remove DNA and impurities, achieving maximum RNA yields with approximately 135.88 ng/µL concentrations. The purity of the extracted RNA was consistently high, with absorbance ratios ranging from 1.9 to 2.0 [17]. Although they obtained poor results in the initial tests, the amount and quality of extracted RNA increased by improving their method. In some other studies, RNA was obtained from the whole body of adult or larval zebrafish using commercial kits or TRIzol [18-20]. In this study, we used an optimized TRIzol-based method to extract RNA directly from the brain of zebrafish larvae. Although it is much more challenging to isolate and extract from the larval brain than the adult zebrafish brain, the results obtained from this method show success. In this study, we used 3 other methods to compare our work. The amount of RNA extracted in this optimized method significantly increased compared to the other 3 methods. The quality of extracted RNA using the optimized method was at the standard level, with minimal protein and polysaccharide concentrations, similar to commercial kits. Still, the amount of extracted RNA was much higher than commercial kits. The results of Real-time PCR on the extracted samples showed the high quality and quantity of RNA extracted using the optimized method. Notably, the optimized TRIzol-based method employed in this study has proven effective in isolating RNA from the challenging larval zebrafish brain, yielding higher quantities of RNA with quality comparable to commercial kits. This advancement enhances the reliability of downstream applications, such as real-time PCR.

## 5. Conclusion

Finally, it can be concluded that the RNA extracted by this method is of high quality and integrity for downstream molecular techniques, including real-time PCR.

## Abbreviations

| | |
|---|---|
| *gapdh* | glyceraldehyde-3-phosphate dehydrogenase |
| *bdnf* | brain-derived neurotrophic factor |
| *chd8* | chromodomain helicase dna binding protein 8 |
| *lrp6* | ldl receptor related protein 6 |
| CNS | Central Nervous System |
| PCR | Polymerase Chain Reaction |

**Acknowledgments**

The authors would like to thank Ms. Firooze. Dara for improving the use of English in the manuscript.

**Author Contributions**

All of the authors contributed substantially to the concept and design of the study. Material preparation, data collection, and analysis were performed by Ma.D., Z.K., Me.D., and H.A. The primary draft of the manuscript was written by Ma.D. All authors read and approved the final manuscript.

**Funding**

This project was supported by Shiraz university of medical science.

**Competing Interests**

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

**Data Availability Statement**

Data is available flowing request from corresponding author.

**References**

1. Briggs JP. The zebrafish: A new model organism for integrative physiology. Am J Physiol Regul Integr Comp Physiol. 2002; 282: R3-R9.
2. Ruzicka L, Bradford YM, Frazer K, Howe DG, Paddock H, Ramachandran S, et al. ZFIN, the zebrafish model organism database: Updates and new directions. Genesis. 2015; 53: 498-509.
3. Howe DG, Bradford YM, Eagle A, Fashena D, Frazer K, Kalita P, et al. The Zebrafish Model Organism Database: New support for human disease models, mutation details, gene expression phenotypes and searching. Nucleic Acids Res. 2017; 45: D758-D768.
4. Liu K, Petree C, Requena T, Varshney P, Varshney GK. Expanding the CRISPR toolbox in zebrafish for studying development and disease. Front Cell Dev Biol. 2019; 7: 13.
5. Zambusi A, Ninkovic J. Regeneration of the central nervous system-principles from brain regeneration in adult zebrafish. World J Stem Cells. 2020; 12: 8.
6. Cayuela ML, Claes KB, Ferreira MG, Henriques CM, van Eeden F, Varga M, et al. The zebrafish as an emerging model to study DNA damage in aging, cancer and other diseases. Front Cell Dev Biol. 2019; 6: 178.
7. Pandelides Z, Thornton C, Faruque AS, Whitehead AP, Willett KL, Ashpole NM. Developmental exposure to cannabidiol (CBD) alters longevity and health span of zebrafish (*Danio rerio*). GeroScience. 2020; 42: 785-800.
8. Babin PJ, Goizet C, Raldúa D. Zebrafish models of human motor neuron diseases: Advantages and limitations. Prog Neurobiol. 2014; 118: 36-58.

9. Truong L, Harper SL, Tanguay RL. Evaluation of embryotoxicity using the zebrafish model. In: Drug safety evaluation: Methods and protocols. Humana Press; 2011. pp. 271-279.
10. Rothenbücher TS, Ledin J, Gibbs D, Engqvist H, Persson C, Hulsart-Billström G. Zebrafish embryo as a replacement model for initial biocompatibility studies of biomaterials and drug delivery systems. Acta Biomater. 2019; 100: 235-243.
11. Robea MA, Balmus IM, Ciobica A, Strungaru S, Plavan G, Gorgan LD, et al. Parkinson's disease-induced zebrafish models: Focussing on oxidative stress implications and sleep processes. Oxidative Med Cell Longev. 2020; 2020: 1370837.
12. Sanabria-Castro A, Flores-Díaz M, Alape-Girón A. Biological models in multiple sclerosis. Neurosci Res. 2020; 98: 491-508.
13. Fu J, Tan YX, Gong Z, Bae S. The toxic effect of triclosan and methyl-triclosan on biological pathways revealed by metabolomics and gene expression in zebrafish embryos. Ecotoxicol Environ Saf. 2020; 189: 110039.
14. Choo BK, Kundap UP, bin Johan Arief MF, Kumari Y, Yap JL, Wong CP, et al. Effect of newer anti-epileptic drugs (AEDs) on the cognitive status in pentylenetetrazol induced seizures in a zebrafish model. Prog Neuro Psychopharmacol Biol Psychiatry. 2019; 92: 483-493.
15. Mazzolini J, Chia K, Sieger D. Isolation and RNA extraction of neurons, macrophages and microglia from larval zebrafish brains. J Vis Exp. 2018. doi: 10.3791/57431.
16. Xiao C, Wang C, Zhang Q, Yang X, Huang S, Luo Y, et al. Transcriptomic analysis of adult zebrafish heart and brain in response to 2,6-dichloro-1,4-benzoquinone exposure. Ecotoxicol Environ Saf. 2021; 226: 112835.
17. Shukla S, Jhamtani R, Dahiya M, Agarwal R. A novel method to achieve high yield of total RNA from zebrafish for expression studies. Int J Bioassays. 2017; 6: 5383-5385.
18. Ujibe K, Nishimura K, Kashima M, Hirata H. Direct-TRI: High-throughput RNA-extracting method for all stages of zebrafish development. Bio Protoc. 2021; 11: e4136.
19. Lan CC, Tang R, San Leong IU, Love DR. Quantitative real-time RT-PCR (qRT-PCR) of zebrafish transcripts: Optimization of RNA extraction, quality control considerations, and data analysis. Cold Spring Harb Protoc. 2009; 2009: pdb-rot5314.
20. Peterson SM, Freeman JL. RNA isolation from embryonic zebrafish and cDNA synthesis for gene expression analysis. J Vis Exp. 2009. doi: 10.3791/1470.