White Paper

Preserving Tissue RNA at Ambient Environment

SUMMARY

Bio-specimen stability is a challenge in life science research and biotechnology, especially when it comes to storing and shipping samples.

RNA sequencing is a powerful tool in the world of biotech and medicine. It's helped unlock many secrets about how genes function within cells; however, there are still some limitations because RNA samples degrade over time when not properly stored or handled which compromises results obtained from that particular experiment/analysis.

With the wider implementation of RNA-sequencing, there has been an increasing demand for reliable ways that protect tissue RNA from degradation over time.

Truckee Applied Genomics (TAG) presents a solution for stabilizing RNA in tissues and maintaining softness for morphological studies by a proprietary formulation. TAG technology allows researchers to store and transport samples at room temperature until ready for analysis, saving time and money while ensuring consistent results from sample to sample.

INTRODUCTION:

Thanks to advancements in multi-omics technology, much of the current research and molecular profiling assays with genomic and transcriptomic targets provide a wealth of information for the advancements in science and medicine[10]. Thus, it is often necessary to maintain a high quality of different molecular signatures in tissues and cell samples for downstream analysis[11,12,13].

RNA is particularly unstable and prone to degradation by multiple degradation reactions such as RNases and hydrolysis[14]. Storage at -80˚C or under liquid nitrogen is effective at maintaining RNA stability and preventing degradation, but requires costly and large freezers. Though effective, such storage temperatures can be practically and economically impossible when transporting samples or working without such infrastructure. Shipping RNA samples usually requires dry ice, this is costly and can be challenging for air transportation. Thus, there is a significant need for specialized RNA storage transport reagents that are more affordable and user-friendly than the current methods.

To answer this need, several reagent products for preserving tissue RNA during room temperature storage have been developed. However, these reagents are either ineffective or render the samples unusable for morphological and protein studies due to their harsh chemical makeup.

TAG-1™ Tissue Stabilization Reagent

TAG-1 Tissue Stabilization Reagent is a ready-to-use reagent that is non-toxic, odorless, and non-crosslinking. Originally designed for molecular pathology, TAG-1 aims to improve the quantity and quality of DNA, RNA, and cellular proteins compared to FFPE tissue while maintaining native morphology and antigenic structures.

TAG-1 protects RNA from degradation by inhibiting nuclease enzymatic activities and stabilizing RNA at ambient temperature against thermodynamic stress and environmental stress, this is achieved by the interplay of components at optimized concentrations.

Background : Current Practices

The most common and reliable method of tissue and molecular preservation is cryo storage in reagents such as TRIzol, flash freezing with liquid nitrogen, or formalin fixation. However, each of these methods has its own distinct disadvantages. TRIzol can be used to maintain RNA at room temperature. However, it requires intense tissue grinding and homogenization that, in combination with its phenol and guanidine isothiocyanate components, prevents its use in histology studies. Conversely, formalin is the most common fixative for whole tissue. However, formalin causes protein and nucleic acid cross-linking that damages and fragmentizes DNA/RNA, thus not suitable for molecular studies. Finally, flash freezing is arguably the most effective method of preserving nucleic acids. Yet, it requires the use of liquid nitrogen or -80 C freezers, thereby making it an impractical method for collecting field samples or for laboratories lacking those resources[9].

Some commercially available reagent products such as RNALater™ , Allprotect™ , and DNA/RNA Shield™ offer additional solutions for maintaining RNA quality at room

temperature. Freshly harvested tissue samples can be submerged in the reagent, allowing it to quickly permeate and protect cellular components. Though the exact chemical composition is not publicly available, these reagents generally have a high salt concentration and/or alcohol concentration which dehydrates the tissue and prevents RNA degradation.

Factors to consider

RNA quality

In molecular assays, high-quality RNA is required to ensure the integrity of results as degraded short nucleotide fragments can lead to erroneous conclusions and compromised downstream applications.

RNA integrity has been evaluated historically by gel electrophoresis followed by imaging, this semi-quantitative methodology is based on the expectation that the mammalian ribosomal RNA bands 28S:18S should resemble a ratio of 2 or above as an indicator of minimal RNA degradation in the sample. However, not all RNA samples contain the 28S and 18S rRNA, alternatively the size distribution of the RNA fragments in the sample can also be used to evaluate the RNA quality.

In recent years, the adaptation of highly sensitive electrophoresis instruments such as the Agilent 2100 Bioanalyzer has standardized the evaluation of RNA quality[1], this allows more consistent measurements across samples and has since been part of best practices in molecular biology, especially in the area of next-generation sequencing.

The electrophoretic RNA measurements from the Agilent instruments could be analyzed for RNA quality in two ways:

RIN number: based on ribosomal RNA ratio, the RNA Integrity Number (RIN) ranges from 1 to 10, with 10 being the most intact and 1 being completely degraded. A RIN ⩾ 7.0 usually satisfies accepted quality requirements for most RNA-Seq protocols[2].

DV200[3]: DV200 evaluates the percentage of fragments that are larger than 200nt, and is independent of the presence of ribosomal RNA in samples. It is reported to be the more reliable predictor of successful NGS library preparation compared to RIN especially in partially degraded

samples such as FFPE[4]. A DV200 greater than 70% indicates high-quality RNA, and samples with values between 50% and 70% are considered to be medium quality[5].

RNA Yield

The yield of RNA from the extraction process is another important factor to consider when it comes to project success and can have a high degree of variability between tissues[6]. The liver, pancreas, and submandibular gland tend to have a higher yield, whereas more fatty and fibrous tissues have the lowest yields. The minimum RNA quantity required could vary depending on the project scope and the types of analysis that require RNA as input, as well as detection sensitivity. For example, the RNA input needed could range between a few hundred nanograms to micrograms for RNA-Seq. However, it is good practice to have a high abundance of RNA samples in case any assay needs to be rerun or additional data needs to be collected.

Compatibility for Multi-omics Study

Integrative multi-omics analysis can offer a more complete understanding of complex biological systems in the research setting as well as translational medicine[7]. It involves the integration, analysis, and interpretation of a combination of molecular profiling data modalities, such as genomics, transcriptomics, and epigenetics, as well as phenotypic or clinical co-variants[8]. This powerful study approach requires the ability to extract different types of molecules (e.g. DNA, RNA, proteins, and metabolites) and obtain additional information that is relevant to specific cases from a single sample. For example, morphological information is generally very valuable in cancer and gene-editing studies. However, such studies are challenging due to the fact that the sample storage conditions could differ across the data type and the optimal conditions for each are not always compatible. For example, tissue preservative reagents specialized for RNA could result in severe tissue shrinkage, making it impossible for phenotypic studies.

Use Cases

TAG-1 Preserves Tissue RNA at Room Temperature

Mouse (c57bl/6) brain tissues were freshly harvested and immediately placed into containers with TAG-1 tissue stabilization reagent, followed by room temperature incubation. At different time points, total RNAs were extracted from tissues, samples that were snap-frozen immediately after harvesting were used as controls.

The RNA quality was assessed by RNA electrophoresis on an Agilent TapeStation 4150 instrument, DV200 RNA quality score was calculated per the manufacturer's instruction.

The result shows that TAG-1 is able to preserve the RNA integrity in mouse brain tissue for up to 72 hours at room temperature, indicated by above 70% DV200 scores across time points.

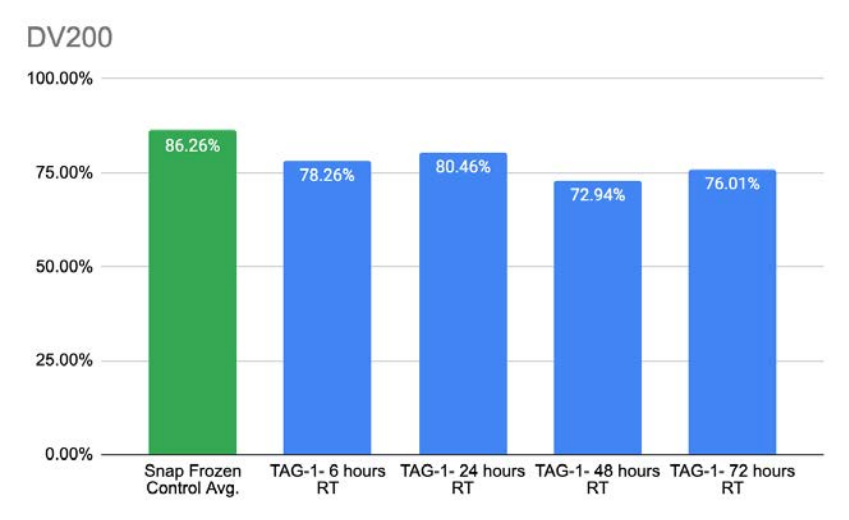

Figure 1: DV200 score of tissue RNAs.

TAG-1 vs RNA later in RNA yield, RNA quality, and Tissue Morphology

Mouse kidney tissues were freshly harvested then placed into either TAG-1 or RNALater, followed by room temperature incubation in parallel. After 6 hours, the morphology of the tissues incubated was observed and compared. Whilst the tissue in TAG-1 remained supple and its original size, the tissue in RNALater appeared to be dehydrated and shrinked to approximately half the size, with clear discoloration.

Figure 2: Mouse kidney tissues incubated(6 hr) in TAG-1 (left) and in RNAlater (right).

RNAs extracted from tissue samples that were snap-frozen immediately after harvesting were used as controls. The purified RNAs were assessed by RNA electrophoresis on an Agilent TapeStation 4150, DV200 RNA quality score was calculated per the manufacturer's instruction. The TAG-1 sample and RNALater samples both returned similar DV200 scores to the snap control samples (92.46%, 91.39%, and 93.47%, respectively). However, it is indicated by the result that RNALater has a lower RNA yield compared to the snap-frozen control and TAG-1 sample. This is possibly partially due to the tissue hardening observed, which could be interfering with the release of RNA from tissues in homogenization.

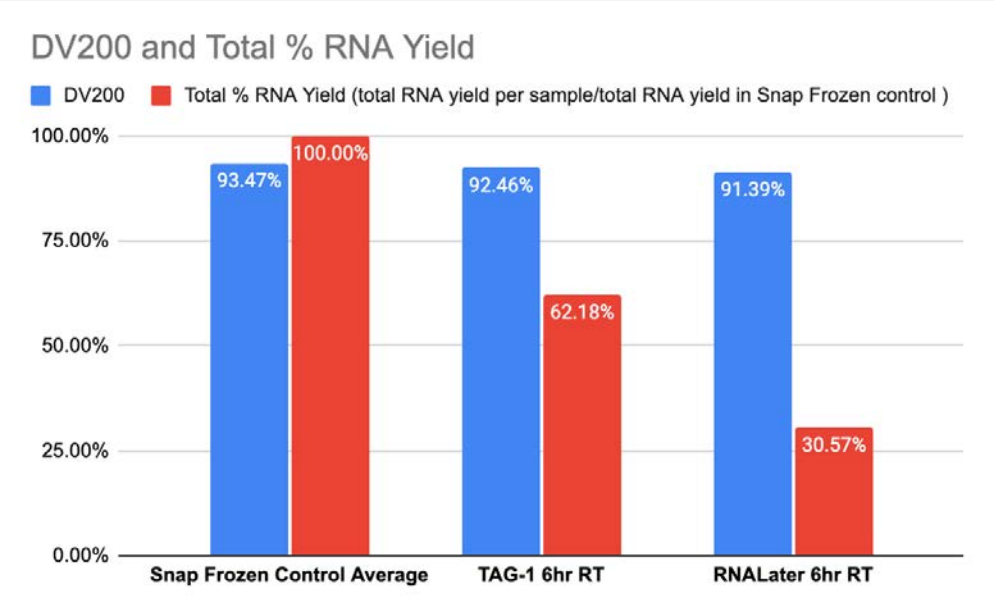

Figure 3: RNA quality and yield in mouse kidney tissue samples.

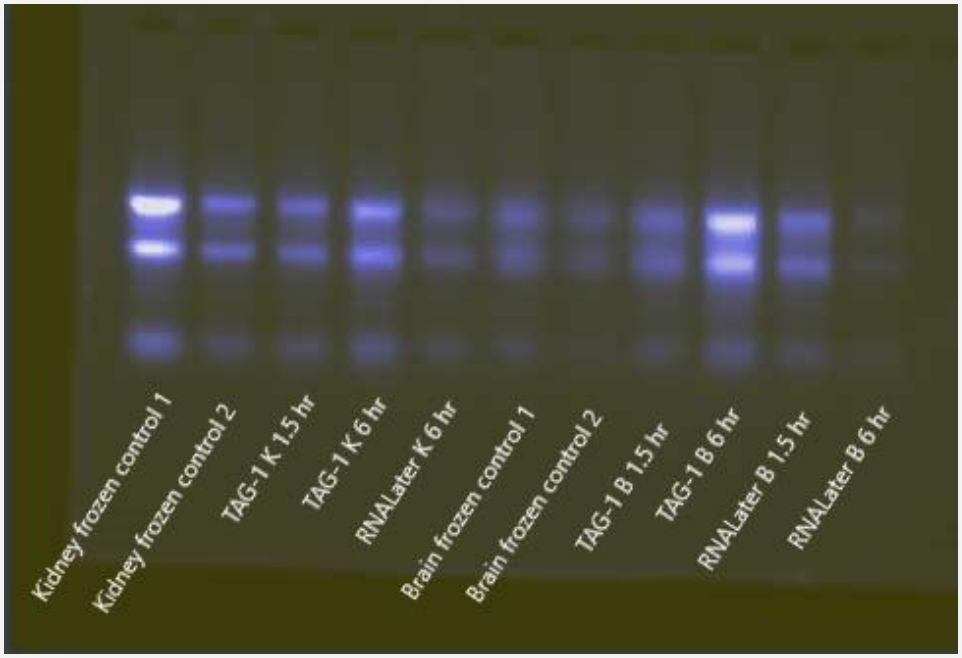

Figure 4: non-denaturing agarose gel electrophoresis of purified total RNA

CONCLUSION

Life science and medicine have advanced greatly in recent years with the development of technologies that can detect multiple molecular analyses and obtain additional phenotypical data from the same sample. This has led to an urgent need for sample transport solutions, which support reliable results from collection sites all over a facility as well unaltered readings when taken at different labs or specialty service providers.

Truckee Applied Genomics has developed a new generation of proprietary reagents that protect and stabilize DNAs, RNAs as well proteins in tissues at ambient temperature. The TAG-1 formulation is nontoxic while also not crosslinking with native tissue structures, making it gentle enough for preserving normal morphological structures.

TAG-1 is available now on Thomas Scientific with a range of packaging options suitable for ready-to-use sample collection and assay-development needs.

This information was provided by Truckee Applied Genomics, an innovative firm based in Nevada dedicated to developing cutting-edge technology solutions that support accurate and reliable molecular testing.

References:

1. Andreas Schroeder, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology* **7**, 3 (2006). https://bmcmolbiol.biomedcentral.com/articles/10.1186/1471-2199-7-3

2. Hugo Roume, et al. Chapter Eleven - Sequential Isolation of Metabolites, RNA, DNA, and Proteins from the Same Unique Sample. *Methods in Enzymology*, **531**, 219-236 (2013). https://www.sciencedirect.com/science/article/pii/B9780124078635000113

3. Takehiro Matsubara, et al. DV200 Index for Assessing RNA Integrity in Next-Generation Sequencing. *BioMed Research International*, **2020** (2020) https://www.hindawi.com/journals/bmri/2020/9349132/

4. illumina. Evaluating RNA Quality from FFPE Samples. *illumina,* **470-2014-001** (2016) https://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/evaluating-rna-quality-from-ffpe-samples-technical-note-470-2014-001.pdf

5. Isabella Wimmer, et al. Systematic evaluation of RNA quality, microarray data reliability and pathway analysis in fresh, fresh frozen and formalin-fixed paraffin-embedded tissue samples. *Scientific Reports*, **8**, 6351 (2018). https://www.nature.com/articles/s41598-018-24781-6

6. Douglas G. Walker, et al. Characterization of RNA isolated from eighteen different human tissues: results from a rapid human autopsy program. *Cell Tissue Bank*, **17**, 3 (2016). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5026224/

7. Ernesto S. Nakayasu, et al. MPLEx: a Robust and Universal Protocol for Single-Sample Integrative Proteomic, Metabolomic, and Lipidomic Analyses. *mSystems*, **1**, 3 (2016).

https://journals.asm.org/doi/10.1128/mSystems.00043-16

8. Sonia Tarazona, et al. Undisclosed, unmet and neglected challenges in multi-omics studies. *Nature Computational Science*, **1**, 395-402 (2021). http://conesalab.org/wp-content/uploads/2021/07/Undisclosed-unmet-and-neglected-challenges-in-multi-omics-studies-1.pdf

9. Mohammad Najafi, Z. S. RNA Preservation and Stabilization. *Biochem. Physiol. Open Access* **03**, (2014).

10. Michal Krassowski, et al. State of the Field in Multi-Omics Research: From Computational Needs to Data Mining and Sharing. *Frontiers in Genetics Systems Biology Archive*, **10**, 3389 (2020). https://www.frontiersin.org/articles/10.3389/fgene.2020.610798/full

11. Sebastian Canzler, et al. Prospects and challenges of multi-omics data integration in toxicology. *Archives of Toxicology*, **94**, 371-388 (2020) https://link.springer.com/article/10.1007/s00204-020-02656-y

12. Jae-Eun Lee, et al. Impact of Preanalytical Variations in Blood-Derived Biospecimens on Omics Studies: Toward Precision Biobanking? *Omics*, **21**, 9 499-508 (2017) https://pubmed.ncbi.nlm.nih.gov/28873014/

13. Luz Ruiz-Godoy, et al. Identification of specific pre-analytical quality control markers in plasma and serum samples. *Analytical Methods*, **17**, 2233-2364 (2019) https://pubs.rsc.org/en/content/articlelanding/2019/AY/C9AY00131J

14. Anne-Lise Fabre, et al. An efficient method for long-term room temperature storage of RNA. *European Journal of Human Genetics*, **22**, 379-385 (2014) https://www.nature.com/articles/ejhg2013145