Application note

Purity Comparison of Automated Extraction Kits for HLA Typing

NanoDrop Eight Spectrophotometer

Human lymphocyte antigen (HLA) markers, which are part of the immune system, help determine whether cells in the human body are self or non-self.[1] Six HLA alleles are inherited from each parent; there are nearly 40,000 human HLA alleles discovered (per the IMGT/HLA Database), making allele combinations unique to individuals.[1-3] HLA typing identifies an individual's HLA alleles and provides important information for blood transfusion or organ transplantation. For example, when a transplant recipient and donor have significant HLA mismatch, this presents as a clinical challenge such as graft-versus-host disease.[1-2,4]

To ensure recipients and donors match alleles through HLA typing, next-generation sequencing (NGS) is used to efficiently provide accurate reads of the alleles; then, bioinformatics is used to compare sequences to the IMGT/HLA database and produce an HLA haplotype snapshot.[5-6] For NGS to be efficient and accurate, the nucleic acid starting material must be free of contaminating salts. High concentrations of salt can interfere with a sequencing reaction by disrupting hydrophobic bonds, ultimately denaturing enzymes needed for sequencing.[7-9]

Ultraviolet-visible (UV-Vis) spectrophotometry is a trusted method for determining nucleic acid purity and detecting salt contamination. Nucleic acids display a spectral peak at 260 nm and a trough at 230 nm in the ultraviolet range (Figure 1). The ratio of the absorbance at 260 nm to 230 nm is referred to as the A260/A230 purity ratio and is calculated to provide a nucleic acid purity estimate.

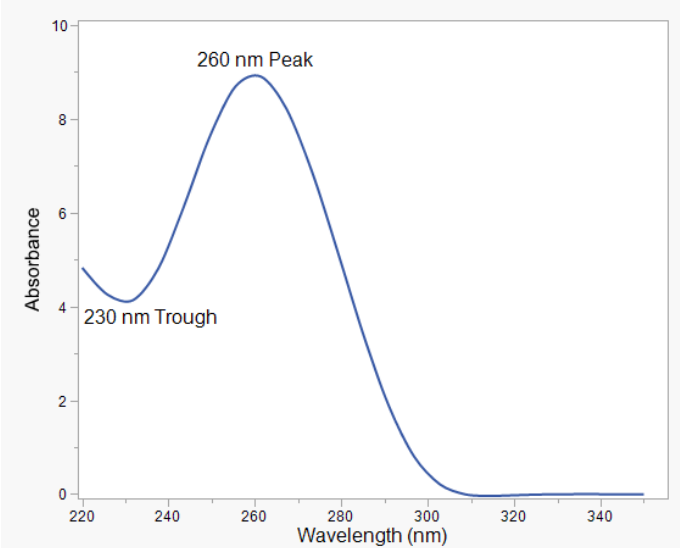

Figure 1. Spectral signature of dsDNA displaying the characteristic peak at 260 nm and trough at 230 nm.

For "pure" dsDNA and RNA, the typical A260/A230 purity ratio range is 2.0 – 2.2. Salts that are used in extraction protocols or in elution buffers typically absorb at or below 240 nm. The increase in absorbance around 240 nm presents as a lower than expected A260/A230 ratio. The A260/A280 ratio is another important indicator of nucleic acid purity as proteins are highly absorbing at 280 nm. For "pure" dsDNA and RNA, this ratio should be close to 1.8 and 2.0, respectively.

To make purity ratio analysis easier, the software designed for use with the Thermo Scientific™ NanoDrop™ Eight Microvolume UV-Vis Spectrophotometer incorporates on-demand technical support for atypical purity ratios with the Thermo Scientific™ Acclaro™ Sample Intelligence technology.

UV-Vis spectrophotometry is also a useful tool for analyzing the composition of elution buffers and their suitability for use in downstream workflows. Some nucleic acid extraction kits include elution buffers that are highly absorbing below 240 nm, indicating the possible presence of high salt concentrations. It is common to see high salt buffers included in extraction kits since certain salts stabilize nucleic acids for storage.[10] However, when working with a UV-Vis instrument, if the spectrophotometer is blanked with a high absorbing salt buffer, the light is attenuated and compromises the nucleic acid measurement accuracy. This is especially true if the buffer is absorbing at the analysis wavelength, which is 260 nm for nucleic acids. The absorption spectrum of the buffer can quickly be analyzed by performing a blank with deionized (DI) water then measuring the buffer as a sample. If the buffer's absorbance at the analysis wavelength is greater than ± 0.04 absorbance units (AU) at a 1.0 cm pathlength, this is generally not an acceptable buffer for UV-Vis absorbance measurements.

Experimental Procedures

Genomic DNA was extracted from 4 whole blood sample pools in duplicate using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit with the Thermo Scientific™ KingFisher™ Apex Benchtop Purification System, using a sample input volume of 2.0 mL and 200 µL elution volume. Genomic DNA was also extracted from 10 whole blood samples in duplicate with competitor "Kit C" and "Instrument C" using a sample input of 200 µL and elution volume of 100 µL. All extracted DNA samples were analyzed for purity and concentration using 2.0 µL volumes on the NanoDrop Eight spectrophotometer and the dsDNA application in the PC software. The NanoDrop Eight instrument was blanked with 2.0 µL of the elution buffer supplied with each kit.

Sample purity was assessed through the A260/A230 and A260/A280 purity ratios reported by the NanoDrop Eight spectrophotometer.

The clean elution buffers supplied with the MagMAX kit and competitor Kit C were tested for NGS and UV-Vis suitability with the dsDNA application in the NanoDrop Eight PC software. The spectrophotometer was blanked with DI water then the elution buffers were measured as samples using 2.0 µL sample volumes.

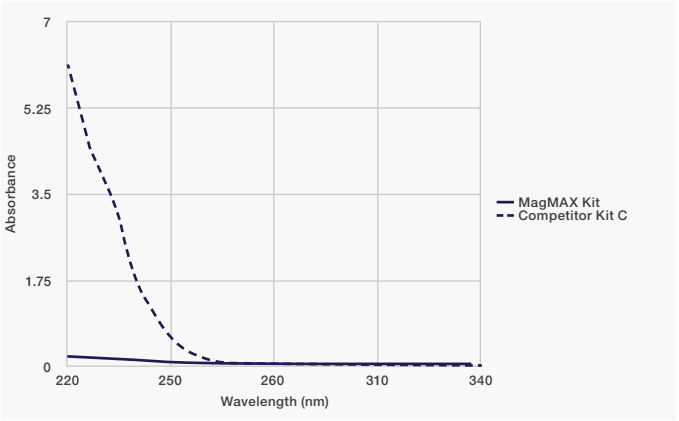

Figure 2. Spectra of clean elution buffers from the MagMAX kit (solid line) and competitor Kit C (dashed line)

Results

The clean elution buffer from competitor Kit C exhibited high absorbance below 260 nm while the clean elution buffer from the MagMAX kit displayed little to no absorbance across the spectral range (Figure 2). The absorbance spectrum for the competitor Kit C elution buffer indicates the Kit C buffer solution contains a salt concentration that may interfere with results from UV-Vis spectrophotometry or NGS.

The concentration and purity results from the DNA extraction for both the MagMAX kit and competitor Kit C are outlined in Table 1. The concentration range from the MagMAX kit was 296 – 893 ng/µL while the competitor Kit C concentration range was 2.5 – 50 ng/µL. The variable concentration ranges can be attributed to the inherent homogeneity discrepancies in whole blood sample pools.

	MagMAX			Kit C		
	Concentration (ng/µL)	A260/A280	A260/A230	Concentration (ng/µL)	A260/A280	260/A230
Average	468.74	1.85	2.10	27.96	1.72	-0.42
Range	296 – 893	1.82 – 1.87	1.75 – 2.39	2.5 – 50	1.58 – 2.03	-0.87 – -0.049

Table 1. Genomic DNA concentration and purity results from all samples extracted with the MagMAX kit (n = 8) and competitor Kit C (n = 20) measured on the NanoDrop Eight instrument.

The MagMAX kit extracted dsDNA at a concentration 17-fold higher than the competitor Kit C and at a higher elution volume (200 µL vs 100 µL), providing more material for downstream experiments.

The A260/A230 purity ratios of samples extracted with competitor Kit C are well below the acceptable threshold of about 2.0 – 2.2 (Figure 3). All the competitor Kit C samples displayed negative A260/A230 ratios due to the high absorbance below 260 nm exhibited by the elution buffer in Figure 2. Since the elution buffer is highly absorbing and was used as a blank, the resulting sample spectrum appears to form a mirror image of the expected buffer spectrum from Figure 2. This result confirms the competitor Kit C elution buffer is not suitable for use with a UV-Vis spectrophotometer. The high salt content also may impact downstream NGS.

In addition to the A260/A230 purity ratio, the A260/A280 purity ratio for the samples extracted with competitor Kit C, with a purity ratio range of 1.58 – 2.03, indicates the presence of contamination by RNA; purity ratio values near 2.0 are indicative that contaminant RNA is present among the dsDNA. The A260/A280 ratios for the MagMAX kit indicate "pure" dsDNA with a range of 1.82 – 1.87.

Conclusions

To accurately perform HLA typing, extracted nucleic acids must be pure to prevent failure of NGS brought about by denatured enzymes. The MagMAX DNA Multi-Sample Ultra 2.0 kit on the KingFisher Apex system has been shown to extract DNA at a high concentration and with excellent purity, as evidenced by the A260/A280 and A260/A230 purity ratios measured on the NanoDrop Eight spectrophotometer. Competitor Kit C and Instrument C, however, did not extract DNA with the desired purity due to the high concentration of salt in the elution buffer, which inhibits downstream NGS and affects accuracy of UV-Vis spectrophotometry measurements. The NanoDrop Eight spectrophotometer was able to provide a purity check of 8 samples simultaneously, allowing quick evaluations of sample purity and buffer suitability to ensure successful HLA typing.

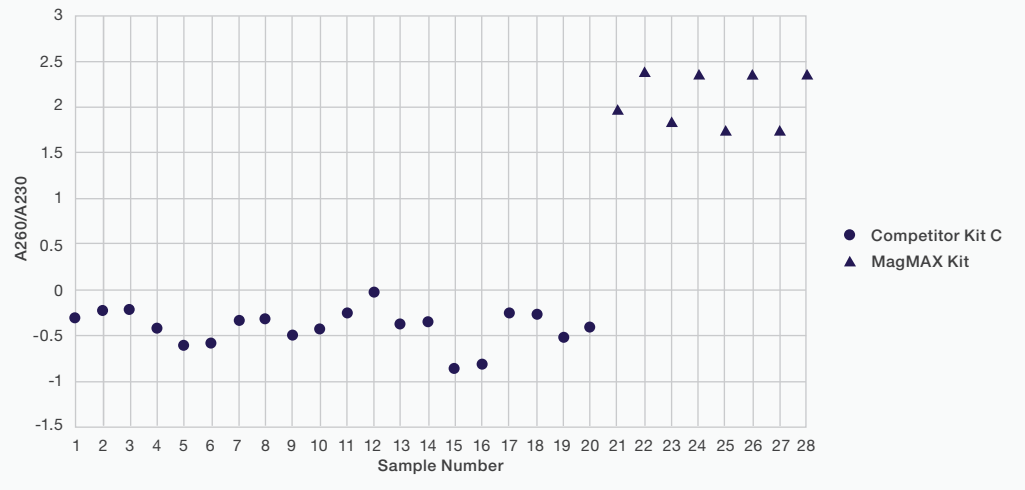

Figure 3. Purity ratio assessment using the A260/A230 ratio from DNA samples extracted with competitor Kit C (circles) and the MagMAX kit (triangles).

References

1. Abbas, A. K., Lichtman, A. H., & Pillai, S. (2014). Basic Immunology: Function and Disorders of the Immune System (Fourth Edition). Saunders, an imprint of Elsevier Inc.
2. Choo, S. Y. (2007). The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications. Yonsei Medical Journal, 48(1), 11–23. https://doi.org/10.3349/ymj.2007.48.1.11
3. Robinson, J., Halliwell, J. A., McWilliam, H., Lopez, R., Parham, P., & Marsh, S. G. E. (2013). The IMGT/HLA database. Nucleic Acids Research, 41(Database issue), D1222–D1227. https://doi.org/10.1093/nar/gks949
4. Petersdorf, E. W. (2017). Role of major histocompatibility complex variation in graft-versus-host disease after hematopoietic cell transplantation. F1000Research, 6, 617. https://doi.org/10.12688/f1000research.10990.1
5. Carapito, R., Radosavljevic, M., & Bahram, S. (2016). Next-Generation Sequencing of the HLA locus: Methods and impacts on HLA typing, population genetics and disease association studies. Human Immunology, 77(11), 1016–1023. https://doi.org/10.1016/j.humimm.2016.04.002
6. Next-Generation Sequencing for HLA Genotyping. College of American Pathologists. Retrieved November 2023, from https://www.cap.org/member-resources/articles/next-generation-sequencing-for-hla-genotyping
7. Sinha, R., & Khare, S. K. (2014). Protective role of salt in catalysis and maintaining structure of halophilic proteins against denaturation. Frontiers in Microbiology, 5. https://www.frontiersin.org/articles/10.3389/fmicb.2014.00165
8. Healey, A., Furtado, A., Cooper, T., & Henry, R. J. (2014). Protocol: A simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. Plant Methods, 10(1), 21. https://doi.org/10.1186/1746-4811-10-21
9. Salvi, G., De Los Rios, P., & Vendruscolo, M. (2005). Effective interactions between chaotropic agents and proteins. Proteins, 61(3), 492–499. https://doi.org/10.1002/prot.20626
10. Tan, Z.-J., & Chen, S.-J. (2006). Nucleic acid helix stability: Effects of salt concentration, cation valence and size, and chain length. Biophysical Journal, 90(4), 1175–1190. https://doi.org/10.1529/biophysj.105.070904

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