Chondrex, Inc.

DNA Assay Kit

Catalog # 6023

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

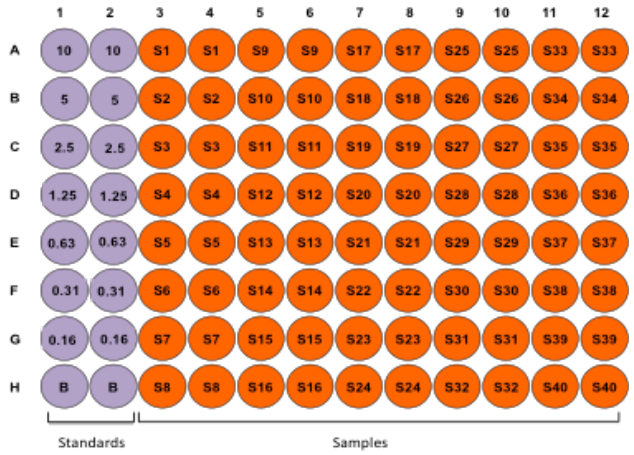
Deoxyribonucleic acid (DNA) is a distinct cell component, therefore DNA amount is correlated with cell number (1). For example, in cartilage tissue engineering, artificial cartilage quality is evaluated by DNA amounts translated as chondrocyte numbers, as well as amounts of collagen and glycosaminoglycans (GAGs) in extracellular matrix components (ECM)(2, 3). DNA can be quantified by the ratio of absorbance at 260 nm and 280 nm, but this method is affected by contaminating proteins, RNA, and chemicals in the sample, requiring additional DNA isolation steps. For tissue analysis, Chondrex provides an alternative DNA assay kit employing the Hoechst 33258 fluorescent dye which specifically binds to Adenine-Thymine base pairs, resulting in fluorescence at excitation 360 nm/emission 460 nm. Since the dye-DNA binding and the fluorescence intensity are unaffected by contaminating proteins and other substances in an optimized assay condition, this DNA assay kit works accurately with samples containing other analytes.

Chondrex also provides native collagen detection ELISAs (Catalog # 6012-6015, 6018 and 6021), Hydroxyproline assay kit (Catalog # 6017), Sirius red/ fast green staining kit (Catalog # 9046), Sirius red total collagen detection kit (Catalog # 9062) and GAGs assay kit (Catalog # 6022). These kits will facilitate further analysis of your studies.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Calf thymus DNA standard (60231)	1 vial	100 µg/ml, 100 µl	-20°C
Reaction Solution (60232)	1 bottle	50 ml	-20°C
Detection Solution Concentrate 200X (60233)	1 vial	50 µl	-20°C
ELISA Black Plate	1 each	96-well	-20°C

Figure 1- A Standard Assay Layout



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NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Partially used reagents may be kept at –20°C.

Note 3: This kit contains components of animal origin from non-infectious animals, but should be treated as potential biohazards in use and for disposal.

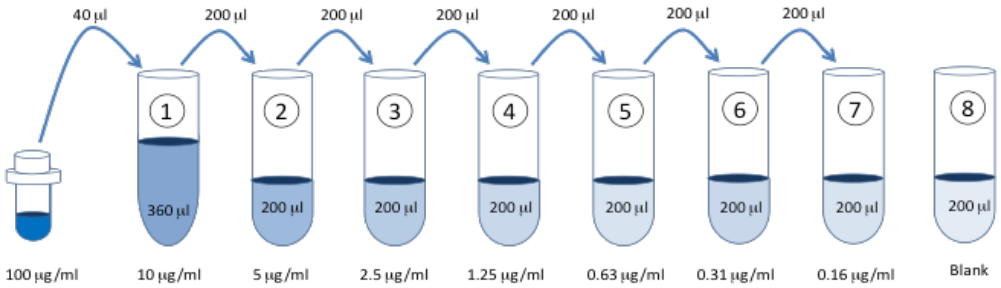
Note 4: Detergents and chemicals used for solubilizing tissues will affect this DNA assay. For accurate results, the solubilization reagent levels in samples should be below the concentrations listed in the table. Please dilute samples with Reaction Solution.

Table 1 - Solubilization reagents in the DNA assay

Reagents	Concentration in samples
Guanidine	0.5 M
SDS	0.025 %
Tween 20	0.0025 %
Triton X-100	0.025 %

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: Take 40 µl of DNA Standard Solution and mix with 360 µl of Reaction Solution (10 µg/ml). Then serially dilute it with Reaction Solution. For example, mix 200 µl of the standard (10 µg/ml) with an equal volume of Reaction Solution to make a 5 µg/ml solution, and then repeat it five more times for 2.5, 1.3, 0.63 0.31 and 0.16 µg/ml solutions. We recommend making fresh serial dilutions for each assay.



2. **Prepare Sample Dilutions**: Dilute samples with Reaction Solution. The optimal sample dilution depends on the sample and preparation methods; therefore we recommend multiple dilutions as a preliminary study.

3. **Prepare 1X Detection Solution**: Mix 5 µl of 200X Concentrated Detection Solution and 1 ml Reaction Solution for each well. For example, 8 samples, 7 point standards, one blank (all in duplicate) will require 1.6 ml of the 1X Detection Solution. Mix 8 µl of 200X Concentrated Detection Solution and 1.6 ml Reaction Solution.

   Note: Prepare the 1X solution just before use. Do not store and reuse the mixed solution for the next assay.

4. **Add Standards and Samples**: Add 50 µl of diluted standards and samples into wells.

5. **Add 1X Detection Solution**: Add 50 µl of 1X detection solution into all wells, and then incubate at room temperature for 5 minutes.

6. **Read plate**: Read the plate at excitation 360 nm/emission 460 nm.

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CALCULATION OF DNA CONCENTRATION

1. Average the duplicate fluorescence intensity (FI) values for the blank (and sample blank), standards, and test samples.
2. Subtract the averaged "blank" (B) FI values from the averaged FI values of standards and test samples.
3. Plot the FI values of the standards against the DNA concentration (µg/ml). Using a log/log plot will linearize the data. Figure 2 shows an example of a DNA standard curve from 0.16-10 µg/ml.
4. The µg/ml of DNA in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the DNA concentration (µg/ml) in the original specimen. Visit www.chondrex.com to download a calculation template for the DNA assay kit.

NOTE 1: DNA levels in samples depend on the sample solubilzation efficiency of individual protocols. consequently, converting DNA levels to cell numbers is not always applicable. Therefore, DNA levels as correlating to cell numbers can be used to compare samples including positive and negative controls.

NOTE 2: To correlate DNA amounts to cell numbers, validation of your DNA solubilization protocol is required. Please refer to the references.

Figure 2 - A typical standard curve for DNA assay

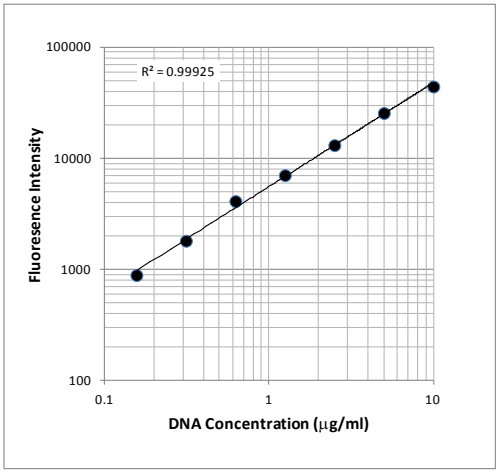

Table 2 - Reproducibility of data assayed by DNA Assay Kit

Test At	0.31 µg/ml	1.3 µg/ml	5 µg/ml
Inter-Assay CV (%)	7.3	5.2	8.0
Intra-Assay CV (%)	7.6	3.6	1.7
Spiking Test*	115.1 %	116.2 %	105.1 %

*Standard was added with known amounts of DNA and then diluted with Reaction Solution for assaying DNA.

REFERENCES

1. V. M. Quent, D. Loessner, T. Friis, J. C. Reichert, D. W. Hutmacher, Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. J Cell Mol Med 14, 1003-1013 (2010).
2. J. H. Yoon, J. Halper, Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact 5, 22-34 (2005).
3. J. A. Buckwalter, H. J. Mankin, Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect 47, 477-486 (1998).

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