Rev. 0

Human Nuclear DNA Damage Quantification qPCR Assay Kit (HNDQ)

Catalog #9008
50 reactions

Product Description

Damage to nuclear DNA (nucDNA) is widely considered a key factor in the development of cancer, neurodegenerative disorders, mitochondrial dysfunction, and various age-related conditions. nucDNA damage serves as a meaningful biomarker for assessing the genotoxicity of drugs and environmental toxins. ScienCell's Human Nuclear DNA Damage Quantification qPCR Assay Kit (HNDQ) operates on the principle that various DNA lesions can impede DNA polymerase progression. Consequently, DNA with fewer lesions amplifies more readily than damaged DNA under identical conditions. Damage levels can be quantified in terms of the lesions per kilobase pair using a Poisson distribution of lesions or the percentage of intact nucDNA of the target sample to the control sample. Additionally, our assay allows for tracking DNA repair kinetics by measuring the restoration of target DNA amplification over time following the removal of the DNA-damaging agent. This assay monitors the integrity of nucDNA.

The primer sets (Cat. #9008a and Cat. #9008b) recognizes and amplifies sequences in the most conserved regions on human nucDNA. We utilize 2X LanaRana Long Range PCR Master Mix (cat #MB6098) and Human Long nucDNA Primer Set (Cat. #9008a) to amplify a long 8.1 kb DNA fragment. For amplifying a short 151 bp nucDNA fragment, we use the 2X GoldNStart TaqGreen qPCR Master Mix (Cat. #MB6018a-1) and Human Short nucDNA Primer Set (Cat. # 9008b). Human DNA from Non-Damaged (untreated) and Damaged (UV treated) cells serves as positive and negative controls for the reaction.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR Master Mix, 1 mL	3 vials	-20°C
MB6098	2X LanaRana Long Range PCR Master Mix	1 vial	-20°C
9008a	Human Long nucDNA Primer Set, Lyophilized	1 vial	-20°C
9008b	Human Short nucDNA Primer Set, Lyophilized	1 vial	-20°C
9008c	Nuclease-Free H_2O	10 mL	4°C
9008d	Reference Non-Damaged Human DNA	100 µL	-20°C
9008e	Reference Damaged Human DNA	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Product Name
DNA isolation kit	SpeeDNA Isolation Kit (ScienCell, Cat #MB6918)
genomic DNA template	Customers' samples
qPCR plate or PCR strip	Plate type or PCR strip based on Customers' qPCR Machine Model

Quality Control
The specificity of the nucDNA long primer set is validated by PCR and gel electrophoresis. The specificity of the nucDNA short primer set is validated by qPCR with melt curve analysis and gel electrophoresis. The nucDNA damage of Reference Damaged Human DNA is determined by qPCR relative to the Reference Non-Damaged Human DNA.

Product Use
HNDQ is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures. HNDQ can be conducted using genomic DNA from human cultured cells or human tissues.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) in the dark at -20°C in a manual defrost freezer, the 2X LanaRana Long Range PCR Master Mix (cat #MB6098), primers (Cat #9008a and cat #9008b) and the reference DNA samples (Cat #9008d and #9008e) at -20°C in a manual defrost freezer, and the nuclease-free H_2O (Cat #9008c) at 4°C. Once thawed, do NOT refreeze GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1), and keep in the dark at 4°C or on ice at all times.

Procedures

Important: ***Only*** *use polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

1. An overview of the experimental setup is detailed in Figure 1 below.

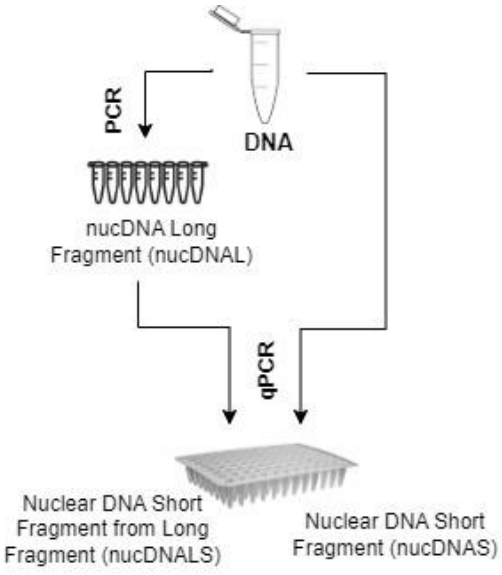

Figure 1- Diagram of the experimental setup. First, perform a PCR amplifying the long nucDNA fragment using the provided Human Long nucDNA Primer Set. Second, perform qPCR using the diluted PCR product with the Human Short nucDNA Primer Set to obtain the Ct value for nucDNALS. Additionally, directly analyze the original DNA samples with qPCR to obtain the Ct value for nucDNAS.

2. **Sample preparation:** Adjust the DNA concentration in all the samples to 10-30 ng/µL. Ensure a consistent amount of DNA loading across all reactions. The same amount of DNA that has been added to the long nucDNA reaction must be added to the short nucDNA reaction since amplification of short nucDNA will be used to normalize the amount of DNA that has been loaded.

3. Allow Cat. #9008a and Cat. #9008b to warm to room temperature.

4. Centrifuge the vials at 1,500x g for 1 minute.

5. Add 200 µl nuclease-free H_2O (Cat. #9008c) to nucDNA long primer set (lyophilized, Cat. #9008a) to make nucDNA long primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

6. Add 600 µl nuclease-free H_2O (Cat. #9008c) to nucDNA short primer set (lyophilized, Cat. #9008b) to make nucDNA short primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

7. Aliquot Reference DNA (Cat. #9008d and Cat. #9008e) as needed. Avoid repeated freeze-and-thaw cycles.

8. For each genomic DNA sample and Reference DNA (Cat. #9008d and Cat. #9008e), prepare a PCR reaction with nucDNA long primer stock solution and 2X LanaRana Long Range PCR Master Mix (Cat. #MB6098). Prepare 20 µl PCR reaction for one well as shown in Table 1.

   Optional: Measure DNA concentration with fluorescent double-strand detection methods for greater accuracy.

   Table 1. Long nucDNA PCR reaction

DNA sample (20-60 ng) or Reference DNA (Cat # 9008d or Cat # 9008e) (2 µl)	x µl
Human Long nucDNA Primer Set stock solution (Cat #9008a)	2 µl
2X LanaRana Long Range PCR Master Mix (Cat #MB6098)	10 µl
Nuclease-free H_2O (Cat #9008c)	8-x µl
Total volume	20 µl

9. Seal the PCR reaction tubes. Centrifuge the tubes at 1,500x g for 15 seconds. Refer to Table 2 for PCR program setup.

   Table 2. Long nucDNA PCR program

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing and extension	68°C	10 min	
Hold	4°C	∞	1

10. Run the PCR program. Then dilute each PCR product 10000x with nuclease free water by serial dilution.

For example, pipette 2 µl of PCR product and mix well with 198 µl of nuclease-free water. Then take 2 µl of this mixture into 198 µl of nuclease-free water to make 10000x dilution. Finally, use 2-5 µl of the diluted PCR product for the subsequent qPCR reaction.

Optional: At this stage PCR products can be analyzed for potential differences in band intensity via gel electrophoresis. The intensity of the band correlates with the amount of intact nucDNA in the sample. Faint band or absence of a band in the sample indicates a high degree of nucDNA damage compared to the control sample (Figure 2). A smeared band may appear above the 8.1 kb band due to the large DNA size and high amount of DNA.

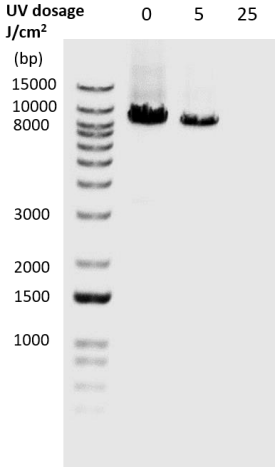

Figure 2. Gel electrophoresis of PCR product.
DNA from Control (Un-Damaged) and UV treated (Damaged) samples tested with the PCR to amplify nucDNAL, 8.1 kb nucDNA fragment. Diluted nucDNAL PCR products were tested with gel electrophoresis.

11. Prepare qPCR reactions for each diluted PCR product, DNA sample and Reference samples (Cat. #9008d and cat #9008e), in triplicate with nucDNA short primer set and GoldNStart TaqGreen qPCR master mix. qPCR reaction (20 µl) for one well is shown in Table 3.

    Note: This kit provides materials for 50 Long nucDNA PCR reactions and 300 Short nucDNA qPCR reactions. We strongly recommend performing the Long nucDNA reaction in duplicate for each sample. For maximum reliability, run Short nucDNA qPCR **in triplicate** for diluted PCR product and original DNA. So, each sample run for Long nucDNA reaction requires 6 reactions of Short nucDNA reaction.

    Table 3. Short nucDNA qPCR Reaction

DNA sample or diluted PCR product	x µl
Human short nucDNA Primer Set Stock Solution (Cat#9008b)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8948c)	8-x µl
Total volume	***20 µl***

12. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.

13. Refer to Table 4 for qPCR program setup. The 2X GoldNStart TaqGreen qPCR master mix (Cat. #MB6018a-1) contains SYBR®Green as the reporter dye and does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

Table 4. Short nucDNA qPCR program

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	32
Annealing	66°C	15 sec	
Extension	72°C	45 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1

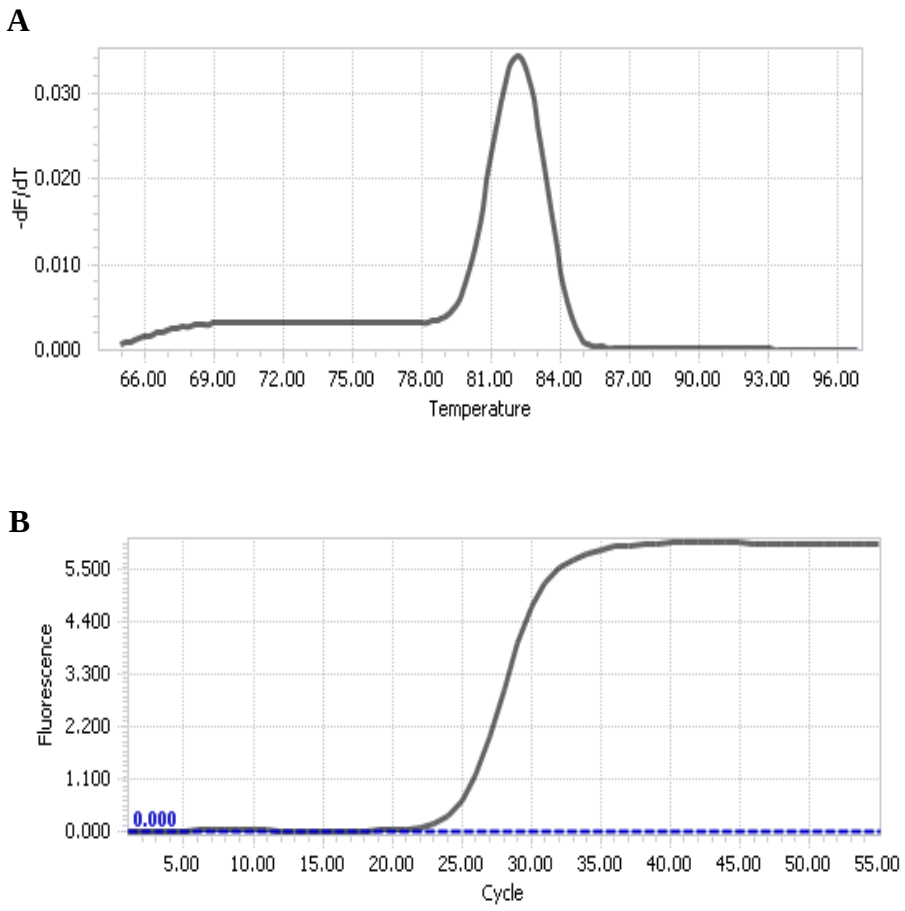

Figure 3. A. A typical amplification curve showing the amplification of a qPCR product. **B**. A typical melting peak of a qPCR product.

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method
Note: Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

1. For ΔCq (nucDNALS) is the quantification cycle number difference of nucDNALS between the damaged (target) and the un-damaged (control) DNA samples.

   ΔCq (nucDNALS) = Cq (nucDNALS, damaged (target) sample) - Cq (nucDNALS, un-damaged (control) sample)

2. For nucDNAS ΔCq (nucDNAS) is the quantification cycle number difference of nucDNAS between the damaged (target) and the un-damaged (control) samples.

   ΔCq (nucDNAS) = Cq (nucDNAS, damaged (target) sample) - Cq (nucDNAS, un-damaged (control) sample)

   Note: the value of ΔCq (nucDNAS and nucDNALS) can be positive, 0, or negative.

3. ΔΔCq = ΔCq (nucDNALS) - ΔCq (nucDNAS)

4. Relative nucDNA damage of the damaged (target) sample to the un-damaged (control) sample (fold)

   $= 2^{-\Delta\Delta Cq}$

5. Percentage of intact nucDNA of the damaged (target) sample to the un-damaged (control) sample

   $= 2^{-\Delta\Delta Cq} / (2^{-\Delta\Delta Cq}+1) \times 100$

6. F= Frequency of lesions per kilo base pair (kb) of DNA
   d= size of the long DNA length being amplified in kilobases (kb). In this kit, nucDNA long fragment is 8.1 kb.

   $F= -\ln (2^{-\Delta\Delta Cq})/d$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of nucDNALS and nucDNAS qPCR obtained from the Human DNA samples, primary cells treated with UV 5 J/cm^{-2}.

Primer set	*Reference Damaged DNA*	*Reference Un-damaged DNA*
nucDNAS	23.69	24.2
nucDNALS	19.32	16.76

1. ΔCq (nucDNAS) = Cq (nucDNAS damaged reference sample) - Cq (nucDNAS, reference undamaged sample)

   = 23.69-24.2

   = -0.51

2. ΔCq (nucDNALS) = Cq (nucDNALS damaged reference sample) - Cq (nucDNALS, reference un-damaged sample)

   = 19.32 – 16.76

   = 2.56

3. ΔΔCq = ΔCq (nucDNALS) - ΔCq (nucDNAS)

   = 2.56- (-0.51)

   = 3.07

4. Relative of intact nucDNA of the damaged reference sample to the un-damaged reference sample (fold)

   = $2^{-\Delta\Delta Cq}$

   = $2^{-(3.07)}$

   = 0.119

5. Percentage of intact nucDNA of the damaged reference sample to the un-damaged reference sample

   = $2^{-\Delta\Delta Cq}/(2^{-\Delta\Delta Cq}+1)x100$

   =0.119/(0.119+1)x100

   =10.6%

6. F= Frequency of lesions per kilo base pair (kb) of DNA

d= size of the long DNA length being amplified in kilobases (kb)

= -ln ($2^{-\Delta\Delta Cq}$)/d

= -ln ($2^{-(3.07)}$)/8.1

= 0.26

Conclusions: In conclusion, for every kilobase of nucDNA there is an estimated 0.26 lesions.