

4D Lifetest™: A highly accurate high throughput comet assay platform

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Introduction

Variability within the Comet Assay (CA) is a major issue, due to differences in parameters within CA protocols, such as agarose concentration, time of unwinding, electrophoresis duration, voltage gradient, temperature of electrophoretic run, the type of electrophoretic tank used and the lack of adequate biological reference standards (1). These inevitably lead to inter-laboratory differences in results.

AIM: Narrow down the variation within the CA by influencing:

- physical parameters (electrophoresis tank)
- the protocol (handling) and
- standard (biological reference)

4D Lifetec AG have developed the innovative 4D Lifetank™ with the following advantages:

- provides accurate voltage and temperature control
- patented, highly homogenous and controlled electric field
- allows high throughput CA analysis through the development of a 96 well format gel spotting plate (GSP)



The 4D Lifetank™

Conclusions

- By using the 4D Lifetank™, we observed an improved dose response and reduced variability in results.
- By further optimizing the CA protocol, we further improved sensitivity of the CA and further decreased variability.
- By optimizing the biological reference, and running these in the 4D Lifetank™ with an optimized protocol, one can benchmark own biological samples.

Results

Reduced Variation in 4D Lifetank™

a) Spot to Spot Variability

COMMERCIAL SYSTEM					4D Lifetank™				
CELL STANDARD	C0	C1	C2	C3	CELL STANDARD	C0	C1	C2	C3
MEAN	5.88	9.82	10.18	36.20	MEAN	7.93	11.02	22.96	40.60
SD	3.05	6.62	7.00	14.83	SD	1.36	0.95	1.30	3.01
CV	51.88	67.47	68.84	40.97	CV	17.12	8.59	5.65	7.41

b) Plate to Plate Variability

COMMERCIAL SYSTEM					4D Lifetank™				
CELL STANDARD	C0	C1	C2	C3	CELL STANDARD	C0	C1	C2	C3
MEAN	5.88	9.82	10.18	36.20	MEAN	7.93	11.02	22.96	40.60
SD	1.31	1.47	5.38	8.43	SD	1.37	0.49	1.03	2.98
CV	22.27	14.93	52.86	23.27	CV	17.23	4.45	4.49	7.35

c) Day to Day Variability

COMMERCIAL SYSTEM					4D Lifetank™				
CELL STANDARD	C0	C1	C2	C3	CELL STANDARD	C0	C1	C2	C3
MEAN	6.22	10.23	9.37	34.02	MEAN	7.55	11.16	23.10	40.60
SD	1.45	1.77	3.41	9.27	SD	1.62	0.59	0.57	0.01
CV	27.93	19.65	28.96	22.86	CV	18.69	5.51	2.52	0.03

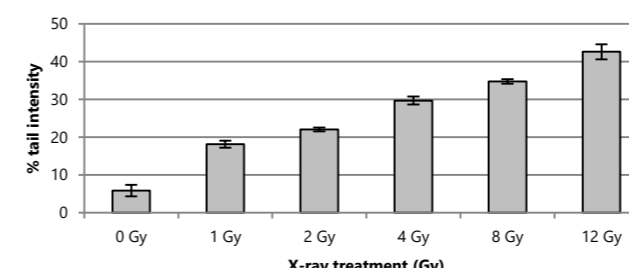
• N = 2, Plates = 3, 6 replicates total
• CV% > 20% are highlighted in red



*Heat map represents increasing DNA damage

Outlook

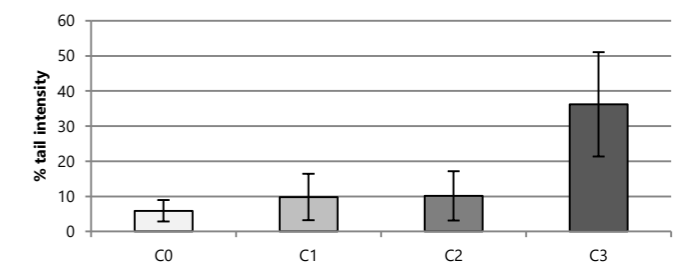
Optimized Biological Reference to Demonstrate Performance



* N = 1, Plates = 2, 4 replicates total

4D Lifetank™ v Commercial System

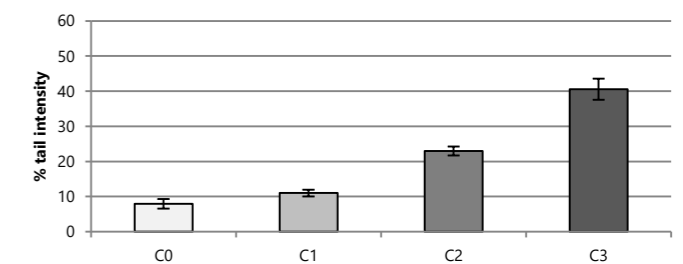
DNA Damage Dose Response from Benchmark Commercial System



Commercial Biological Reference Standard

* N = 2, Plates = 3, 6 replicates total

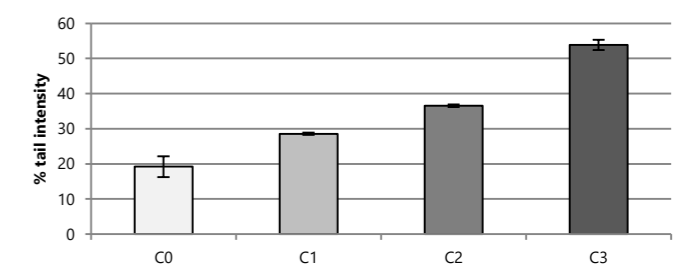
Reduced Variation and Improved Dose Response from 4D Lifetank™



Commercial Biological Reference Standard

* N = 2, Plates = 3, 6 replicates total

Optimized Protocol and Improved Outcome on 4D Lifetank™



Commercial Biological Reference Standard

* N = 1, Plates = 1, 2 replicates total

CELL STANDARD	C0	C1	C2	C3
MEAN	19.22	28.57	36.54	53.88
STDEV	2.97	0.32	0.35	1.45
CV	15.48	1.12	0.97	2.69

Comparative comet assay experimental set up

- **4D Lifetank™ v Commercial system:** To prove the accuracy of the 4D Lifetank™, we chose the benchmark commercial CA electrophoresis unit and ran parallel CAs. Using the commercial reference cell standards C0 (untreated) and C1-C3 (representing treated cells with increasing DNA damage), we ran the CA using the manufacturers recommendations (Table 1). Images were acquired using a Zeiss Axio microscope (100 comets counted per replicate), and analysed using OpenComet plugin for ImageJ software.
- **Variability assessment of the 4D Lifetank™:** To assess for possible CA variability, we compared the mean % tail intensities achieved between: a) spots; b) plates and; c) days the experiment was performed in.
- **Optimized protocol:** We addressed each parameter listed in the flowchart (below) to optimize the 4D Lifetest™ CA protocol (Table 1).
- **4D Lifetest™ optimization:** To assess our optimized CA protocol, we ran the commercial biological reference standards on the 4D Lifetank™ using our improved protocol.
- **Outlook - Optimized biological reference:** As an additional step to increase the range of DNA damage in cells and demonstrate performance of the 4D Lifetank™, we created a set of biological reference standards. Ionizing radiation is a reliable and robust damaging agent, therefore X-ray irradiated A549 cells (0-12 Gy) were used and run on the 4D Lifetank™.

Table 1. Commercial Protocol		4D Lifetest™ Protocol
SCGE unit	Commercial/4D Lifetank™	4D Lifetank™
Cells	Commercial	Commercial
Chemicals	Commercial	4D Lifetest™
Parameters		
Agarose concentration	As protocol	0.40%
Lysis time	Overnight	60 min
Unwinding time	40 min	40 min
Buffer temperature	4C°	15C°
Electrophoresis time	30 min	30 min
Voltage	1.1V/cm	1.1V/cm
Staining	SYBR Gold	SYBR Gold

Sources of Variation in CA and Optimization

