

GeneFiX In-Use Stability Study over 5 years

Method:

Accelerated ageing stability study:

Accelerated ageing studies are widely used to reliably determine the stability of saliva samples stored in a stabilising buffer using the Arrhenius equation to calculate the equivalent storage time at room temperature for a sample held at an elevated temperature. In this case the elevated temperature used was 50°C and the equivalent stability time at 21°C was calculated.

A large saliva sample was collected and an equal volume of GeneFix stabilisation buffer added. After vortexing well to mix, a sample was removed for DNA isolation using the GeneFix Saliva-Prep kit on Day 0 and the remaining stabilised saliva sample was incubated at 50°C for 315 days (equivalent to 5 years at 21°C). Samples were removed at time points equivalent to 1, 2, 3, 4 and 5 years and isolated using the GeneFix Saliva-Prep kit in the same way as the Day 0 sample. All isolated DNA samples were stored at -20°C until the completion of the study.

DNA yields for all 6 samples were measured by Qubit dsDNA BR assays, and whole DNA integrity checked on an agarose gel.

Room temperature stability study:

A second large saliva sample was collected and an equal volume of GeneFix stabilisation buffer added. After vortexing well to mix, a sample was removed for DNA isolation using the GeneFix Saliva-Prep kit on Day 0 and the remaining stabilised saliva sample was incubated at room temperature (20 to 25°C) for 3 years (study ongoing). Samples were removed at time points of 3 months, 6 months, 9 months, 12 months, 18 months, 24 months and 36 months and isolated using the GeneFix Saliva-Prep kit in the same way as the Day 0 sample. All isolated DNA samples were stored at -20°C until the completion of the study.

DNA yields for all 8 samples were measured by Qubit dsDNA BR assays, and whole DNA integrity checked on an agarose gel.

DNA yield results:

Accelerated ageing study

Time point	DNA concentration ng/µl
Day 0	47
Equivalent 1 year	49.1
Equivalent 2 years	42.7
Equivalent 3 years	32.3
Equivalent 4 years	49.4
Equivalent 5 years	43.8

Room Temperature stability study

Time point	DNA concentration ng/µl
Day 0	21.3
3 months	24.8
6 months	25.5
9 months	24.6
12 months	20.4
18 months	27.8
24 months	24.1
36 months	26.1

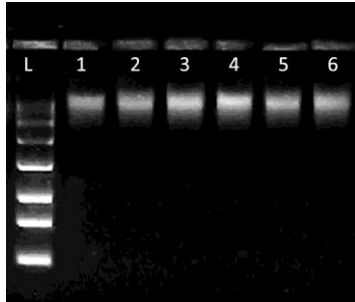
- Yields are maintained over the time period of the study for both the accelerated ageing and room temperature studies.
- Differences in yields between the 2 studies are dependent on the DNA concentration in the original saliva samples which came from 2 different individuals.

AutoGen is the North American supplier for the GeneFiX. Please contact Rob Osborn at [508.395.8161](tel:508.395.8161) or at ROsborn@autogen.com for more information.

Whole DNA on agarose gel:

Accelerated ageing study

1ul of each sample was loaded onto a 2.2% agarose FlashGel, the DNA marker has maximum band size of 4Kb.

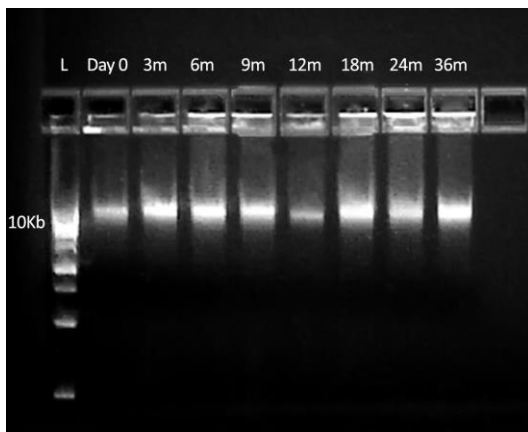


Samples:

- 1: Day 0
- 2: 1 Year equivalent
- 3: 2 Year equivalent
- 4: 3 Year equivalent
- 5: 4 year equivalent
- 6: 5 Year equivalent

Room temperature stability study

1ul of each sample was loaded onto a 2.2% agarose FlashGel, the DNA marker has maximum band size of 10Kb.



- All samples from both the accelerated ageing study and room temperature stability study contain intact non-degraded high molecular weight genomic DNA, with no evidence of degradation or shearing.
- There is no change in the appearance of the DNA from the fresh samples isolated on Day 0 and those stored for either 315 days at 50°C, equivalent to 5 years at room temperature, or those stored at room temperature for 36 months.

To conclude:

DNA in saliva samples collected into GeneFix stabilisation buffer remains stable and undegraded for at least 5 years. Comparing DNA stability at room temperature against DNA stability in an accelerated ageing study at 50°C allows extrapolation of the room temperature stability duration to be determined as at least 5 years.

The GeneFix Saliva-Prep kit (GSPN-50) isolates intact, high molecular weight DNA from saliva stabilised in GeneFix stabilisation buffer, and the resulting DNA samples show no evidence of degradation or RNA contamination.

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