Validation of the use of prefilled wash and elution plates with the MagMAX CORE Nucleic Acid Purification Kit

Purpose

During an outbreak, time is of the utmost importance. Prefilling of plates gives laboratories the flexibility to fill plates when time allows and use the plates throughout the week to reduce time pressure. We evaluated whether wash and elution plates can be prefilled and stored until use with the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit.

For this validation we filled the plates with the standard recommended volumes of MagMAX CORE wash and elution buffer, sealed the plates for up to 8 days, and compared their functionality to that of freshly filled plates.

Methods

1. A foil seal was used to seal full plates containing the following solutions, according to the MagMAX CORE Nucleic Acid Purification Kit user guide (Pub. No. MAN0015944, revision C.0):
   - 500 µL Wash 1 in each well of a Thermo Scientific™ KingFisher™ deep-well plate
   - 500 µL Wash 2 in each well of a Thermo Scientific™ KingFisher™ deep-well plate
   - 90 µL Elution Buffer in each well of a standard Thermo Scientific™ KingFisher™ 96-well microplate
2. The plates were sealed using an adhesive film applicator for PCR plates.
3. The plates were left on the lab bench for 8 days (unstacked) at room temperature according to the storage recommendation (15–30°C).
4. The weights of the plates were recorded over 8 days (excluding weekends).
5. For each isolation, 100 µL of bovine blood spiked with 20,000 copies of Applied Biosystems™ VetMAX™ Xeno™ Internal Positive Control RNA was used.
6. Samples were extracted on the Thermo Scientific™ KingFisher™ Flex Purification System using the "simple" workflow.
7. Quantitative reverse transcription PCR (RT-qPCR) was employed to detect the Xeno RNA, and quantitative PCR (qPCR) to detect a *GAPDH* target in genomic DNA.

Results

After 8 days, the plates containing Wash 1 and Elution Buffer weighed the same (Table 1). The Wash 2 plate lost 0.2 g over 8 days, which is minimal and had no impact on extraction and detection of the RNA and DNA. Real-time PCR showed minimal variation in C_t between samples extracted with fresh vs. prefilled plates (Figures 1–4).

Table 1. Weight of foil-sealed plates left at room temperature for 8 days.

	Weight (g)		
	Wash 1	Wash 2	Elution Buffer
Initial	135.4	129.0	41.7
Day 1	135.4	129.0	41.7
Day 5	135.4	129.0	41.7
Day 7	135.4	128.9	41.7
Day 8	135.4	128.8	41.7

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	1	2	3	4	5	6	7	8	9	10	11	12
A	32.22	32.55	31.24	31.39	30.81	31.10	31.43	31.15	31.13	31.42	32.35	31.59
B	31.41	31.77	31.44	30.97	32.17	30.31	30.68	31.60	32.72	30.79	30.68	33.29
C	32.15	31.79	31.33	34.12	31.30	30.97	31.18	30.72	31.19	30.80	31.28	31.47
D	31.44	31.76	34.12	30.86	30.67	31.43	31.41	30.60	30.96	30.52	30.76	31.83
E	31.65	31.32	31.97	31.89	30.93	31.74	33.03	30.80	30.79	31.09	33.34	31.23
F	31.96	34.34	30.98	31.23	31.40	31.33	31.30	31.47	31.30	31.51	31.72	32.36
G	31.75	31.59	31.78	31.38	31.13	30.99	30.99	31.19	31.48	31.04	31.84	32.51
H	32.09	32.26	32.20	31.40	32.00	31.23	32.00	31.37	31.84	32.23	34.16	31.45

Figure 1. C_t values of real-time PCR of Xeno RNA extracted with reagents in fresh plates. The Xeno RNA internal positive control was used to assess RNA recovery. For samples extracted using plates with fresh reagents, the average C_t was 31.6 (SD = 0.8). Darker colors indicate higher C_t.

	1	2	3	4	5	6	7	8	9	10	11	12
A	31.82	31.60	31.17	31.16	31.24	31.79	32.54	32.08	31.60	31.99	31.95	32.12
B	31.61	31.43	31.58	31.24	31.86	32.09	31.91	31.83	32.40	31.48	32.07	32.98
C	31.90	31.88	32.02	31.98	32.76	33.63	31.65	31.75	32.18	32.01	32.11	32.61
D	32.60	31.72	33.30	32.42	32.96	33.40	32.16	33.99	32.09	32.80	31.95	33.16
E	32.12	31.62	32.07	31.98	31.63	32.36	32.36	32.02	31.60	32.15	32.15	32.97
F	32.44	34.71	32.05	32.30	33.37	32.97	33.25	32.77	32.09	33.67	32.94	33.15
G	33.20	33.12	33.18	32.56	32.77	33.02	33.07	31.82	31.50	32.60	33.39	32.54
H	33.84	33.46	33.63	33.00	33.51	32.35	32.94	32.83	32.34	33.02	33.78	32.70

Figure 2. C_t values of real-time PCR of Xeno RNA extracted with reagents in 8-day-old plates. The average C_t for Xeno RNA extracted using 8-day-old foil-sealed plates was 32.4 (SD = 0.7). When compared to Figure 1, there is minimal variation in Xeno RNA recovery between the foil-sealed plates left at room temperature and fresh plates. Xeno RNA recovery is very consistent across all 96 wells for both plate types.

	1	2	3	4	5	6	7	8	9	10	11	12
A	25.33	26.00	25.20	25.24	24.87	24.75	24.82	24.85	24.98	25.06	25.27	25.89
B	25.23	25.53	24.72	24.67	25.78	24.07	24.74	25.14	25.80	25.11	25.57	25.99
C	25.33	23.89	24.00	26.09	24.59	24.88	24.36	23.73	23.69	24.73	24.77	25.34
D	24.87	24.15	26.02	24.29	24.30	23.94	24.43	24.80	23.90	24.00	24.54	24.56
E	25.48	24.66	24.71	23.48	24.29	NEG	NEG	24.41	24.09	25.07	25.49	25.07
F	25.21	25.73	25.00	24.34	24.52	25.08	24.18	24.24	25.15	25.18	25.81	24.98
G	24.81	24.52	24.87	23.85	25.10	24.31	24.42	25.40	24.74	24.67	24.99	25.28
H	25.37	25.43	25.34	25.12	25.05	25.30	25.04	24.50	24.75	24.79	25.31	25.48

Figure 3. C_t values of real-time PCR of *GAPDH* DNA extracted with reagents in fresh plates. *GAPDH* detection was used to assess DNA recovery from bovine blood. For fresh plates, the average C_t across the 96 wells was 24.9 (SD = 0.6). NEG = negative control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	24.55	25.26	25.57	25.71	25.93	25.90	25.80	25.88	26.09	26.10	25.35	24.99
B	26.27	25.77	25.65	26.28	26.65	25.86	25.84	26.01	25.55	25.37	24.99	24.95
C	24.93	25.28	25.90	26.10	25.82	25.70	25.84	25.81	25.54	25.42	25.35	25.42
D	25.34	25.60	26.03	25.61	25.46	25.78	25.60	25.95	25.05	25.54	24.79	25.72
E	25.21	25.15	26.27	26.14	26.38	NEG	NEG	26.01	26.06	26.07	25.97	25.57
F	26.00	25.60	25.48	26.00	25.88	26.14	26.13	25.74	25.77	25.72	25.94	26.13
G	25.39	25.59	25.35	25.39	25.85	25.49	25.38	25.07	25.64	25.79	26.06	26.04
H	26.01	25.85	25.82	25.63	25.60	25.65	25.11	25.16	25.23	25.99	26.18	26.16

Figure 4. C_t values of real-time PCR of *GAPDH* DNA extracted with reagents in 8-day-old plates. For samples extracted using reagents left in foil-sealed plates for 8 days at room temperature, the average C_t across 96 wells was 25.7 (SD = 0.4). When compared to Figure 3, there is minimal variation in average DNA recovery between samples extracted using foil-sealed plates left at room temperature and fresh plates. NEG = negative control.

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Conclusions

This validation shows that no loss of functionality was observed after 8 days of storing the prefilled plates at room temperature. The underside of the foil film turned white after 8 days. However, this did not affect the quality of the wash and elution buffers. There was no measured change in the weights of the Wash 1 or Elution Buffer plates, and the change in the Wash 2 plate was insignificant. The data show that plates can be filled, sealed, and used up to 8 days later without loss of sensitivity for both an RNA and a DNA target. Plates should be carefully sealed to prevent evaporation around the edges of the plate. Here plates were stored unstacked in order to subject all plates to the same conditions. Stacking plates and placing an empty plate on top may help create even more optimal storage conditions.

Ordering information

Product	Size	Cat. No.
MagMAX CORE Nucleic Acid Purification Kit	100 reactions	A32700
MagMAX CORE Nucleic Acid Purification Kit	500 reactions	A32702
VetMAX Xeno Internal Positive Control RNA	100 reactions	A29763
VetMAX Xeno Internal Positive Control RNA	500 reactions	A29761
Adhesive PCR Plate Foils	100 sheets	AB0626
Adhesive Film Applicator	5 applicators	4333183
KingFisher deep-well plates	50 plates	10045/95040460
KingFisher 96-well microplate	48 plates	97002540
KingFisher Flex Purification System with 96 Deep-Well Head	1 instrument	5400630

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