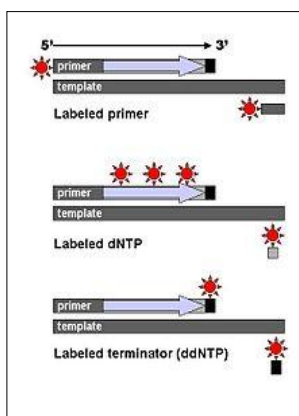


## **MagSi-DNA clean<sup>FIX</sup> kit: All your PCR and sequencing sample purifications on any scale in one single solution!**

**MagnaMedics' one solution approach for PCR clean up and DeoxyDye terminator removal, enables researchers to make flexible use of one kit for many common applications performed in genomic research.**

### **Sanger sequencing technology and MagSi-DNA clean<sup>FIX</sup>**



Fredrick Sanger, invented in 1977 the "dideoxy" chain-termination method for sequencing DNA molecules as still performed in many of the genomic research labs today. The classical chain-termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleotides (dNTPs), and modified nucleotides (dideoxynucleotides) that terminate DNA strand elongation as shown on the left-hand side figure. These ddNTPs nowadays are fluorescently labelled for detection in automated

sequencing machines like the Applied Biosystems® 3730xl. Stretches of over 1000 bases can be analysed. It provides an excellent manner to generate data after isolating the genomic DNA from organisms.

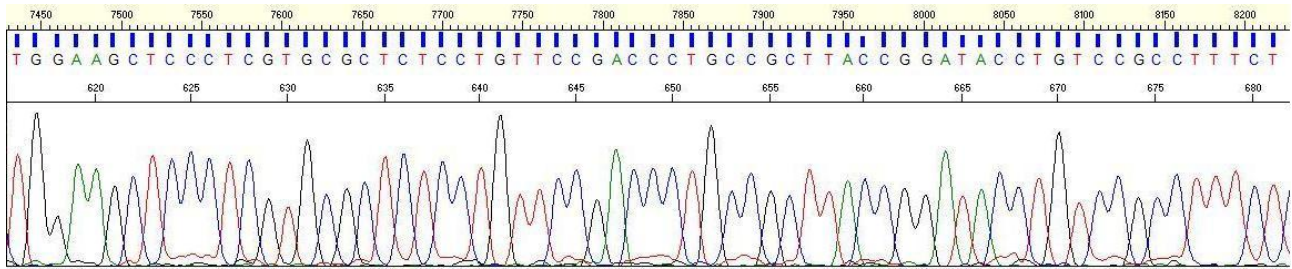


**MagnaMedics Diagnostics BV** provides an excellent solution for helping scientists with the routine procedure of clean up needed in Sanger DNA sequencing. Removal of excess ddNTP is crucial. MagnaMedics has listed the main advantages in the [MagSi-DNA cleanFIX](#) brochure.

**Table 1: Comparison of DNA clean up techniques used for dideoxy dye terminator removal**

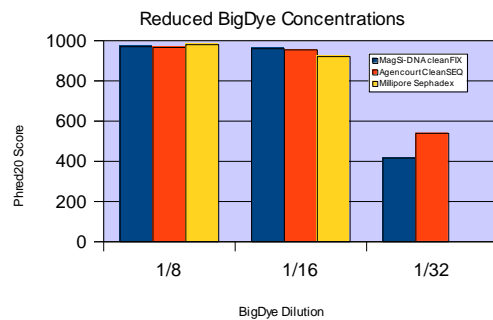
	Processing Time	Multiple sample types	Fully automatable	MTP plate format	steps involved
MagSi-DNA clean <sup>FIX</sup> kit	< 25 minutes*	yes	yes	96, 384, (tube)	no centrifugation, 2 washing step
Other Bead based kits	25 minutes	no	yes	96, 384, (tube)	no centrifugation, 2 washing steps
Column based kits	>15 minutes	yes	yes	96, (tube)	centrifugation or vacuum manifold, 1 washing step
Gel / Sephadex clean-up	> 30 minutes	yes	no	no MTP format possible	centrifugation and multiple washing steps
Ethanol precipitation	> 2 hours	yes	no	96, 384, (tube)	Multiple centrifugation and washing steps

## High quality DNA sequencing readout



**Figure 3:** Data proving the quality of our set-up. Details: 10  $\mu$ L sequence sample, template used: pGEM as provided in ABI Big Dye kit V3.1. Data kindly provided by Dr Jost Muth, Fraunhofer IME institute Aachen.

**MagSi-DNA clean<sup>FIX</sup>** is designed to enable high quality DNA sequencing data readouts. The kit delivers high Phred20 scores (>700 bps) and high sequencing pass rates (>85%). High signal-to-noise ratios allow readout of a wider range of samples than other methods. In addition, the high signal strength allows the user to lower the BigDye concentrations without losing quality of sequencing results as shown in figure 4.



**Figure 4:** 10  $\mu$ L sequencing reactions of pGEM DNA prepared with varying BigDye concentrations were purified using MagnaMedics' MagSi-DNA clean<sup>FIX</sup>, Agencourt CleanSEQ and sephadex method.

Besides removal of dye terminator, many scientist prefer use one kit for all genomic purification processes like are:

- cDNA clean up
- PCR clean up
- DNA concentration
- Amplicon clean up
- Nested PCR clean up

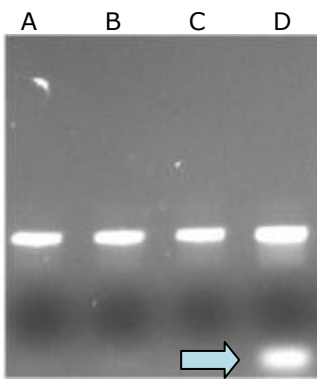
MagnaMedics Diagnostics enables all that with the introduction of **MagSi-DNA clean<sup>FIX</sup>** kit.

## DNA clean up of PCR reactions

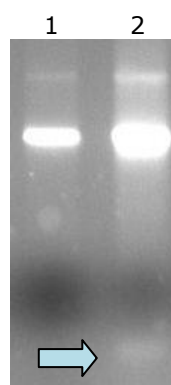
**The MagSi-DNA clean<sup>FIX</sup>** kit is an efficient tool for separating PCR products (>80 bps – 30 kbs) from unwanted products and residues potentially present in the sample as well. It efficiently removes dNTP's, primers, primer dimers, enzymes, additives and salts. The **MagSi-DNA clean<sup>FIX</sup>** kit ensures researchers to manually or automatically prepare their samples with ease. Moreover, the promoted kit can be used for concentrating low amounts of PCR products, amplicons and cDNA as well.

**Recovery of PCR product without unwanted residues**

For PCR downstream applications removal of (single) nucleotides, residual primers and primer dimers is essential. Efficient removal of these contaminants is achieved by adding **binding buffer P**, that is included in the **MagSi-DNA clean<sup>FIX</sup> kit**. Purification up of PCR products between 0,1 and 1,0 kbs, has been demonstrated as shown in figure 5. By using **MagSi-DNA clean<sup>FIX</sup> kit** overall DNA yields up to 75% of the starting amount can be achieved, so without significant loss of signal intensity.

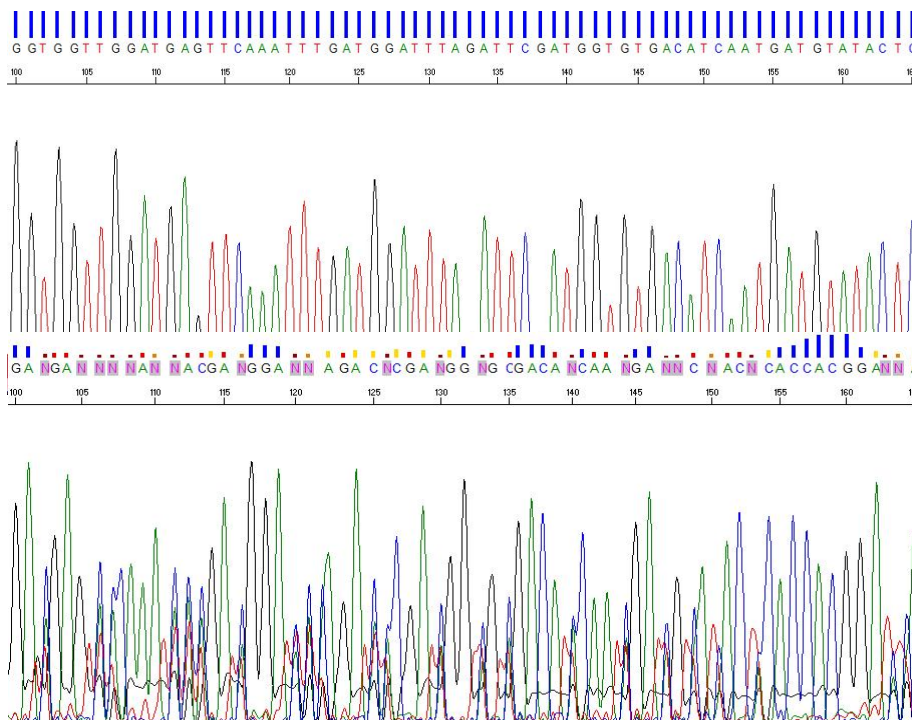


**Figure 5:** A 0,5 kb PCR product was purified with MagSi DNA clean<sup>FIX</sup>. Lanes A – C show the cleaned PCR product with various PCR mastermixes showing universal use of MagSi-DNA clean<sup>FIX</sup>. Lane D shows the crude PCR product including a low molecular (< 100 bps) primer dimer band.



**Figure 6:** pET PCR product purification using MagSi-DNA-clean<sup>FIX</sup> (1) or classic EthOH. precipitation (2). Note that in sample 2 contaminants are present after purification using classic EthOH techniques. These contaminants will cause high noise ratios in the downstream sequence read out as shown with same as demonstrated in figure 7.

A 1 kb PCR product (a potato genomic sequence) was ligated in a pET vector. It was amplified using vector-specific primers, then purified using **MagSi-DNA clean<sup>FIX</sup>** or classic ethanol precipitation (Figure 6). Sequencing these samples using an internal primer allows for read out of 840 bp of the insert DNA. Purification of sequencing reactions was done using Sephadex clean up on both samples. Results show that EtOH sample purification, unlike MagSi DNA clean<sup>FIX</sup> purification, generates very noisy read out signals resulting from residual PCR primers in the sequence sample, with much lower trace scores and read lengths (Figure 7).



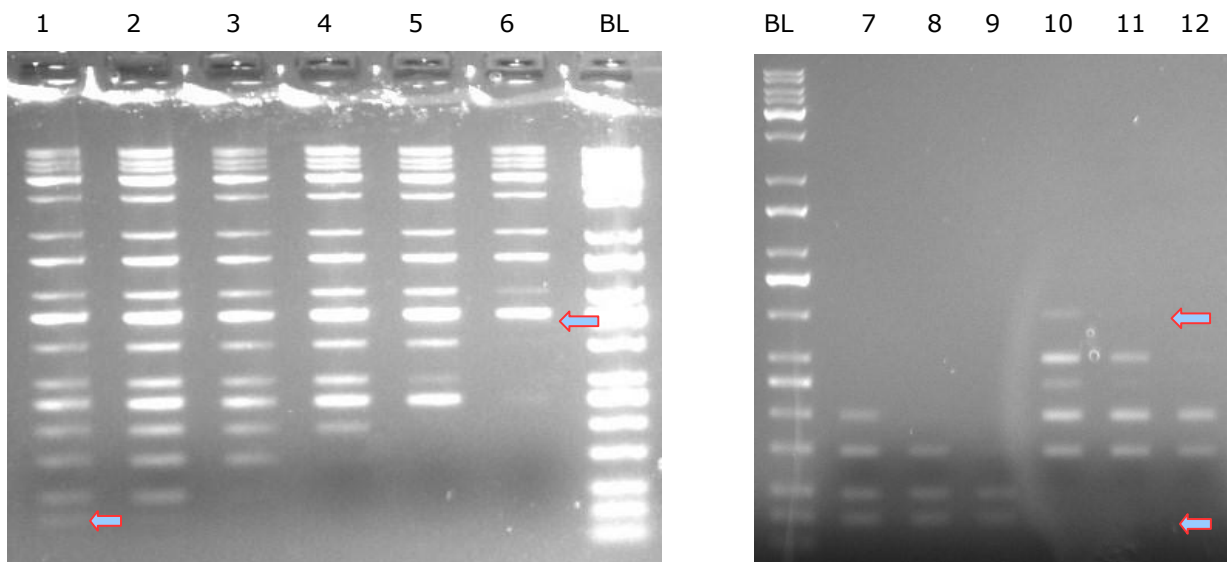
**Figure 7.** Top: Crude PCR product purified using MagSi DNA clean<sup>FIX</sup>. Bottom: EtOH precipitation for cleanup of PCR sample. Both crude cycle sequence reaction samples were cleaned using Sephadex to avoid biases. Sequence reads from bases 100 to 165 of both samples are shown. While using MagSi-DNA clean<sup>FIX</sup> kit, excellent reads are shown (top drawing) while PCR fragments cleaned via EtOH precipitation (bottom drawing) shows many undetermined bases (Ref.: Jost Muth and Raphael Soeur, Fraunhofer Institute IME in Aachen).

## Size fractionation using MagSi-DNA clean<sup>FIX</sup> solutions

Many genomic relevant procedures require purification of DNA fragments for downstream applications (e.g. Next Generation Sequencing (NGS)). Specifically in combination with processes like Chromatin IP (ChIP) and Methylated DNA IP (MeDIP) there is a potential requirement to isolate DNA fragments of specified lengths. Also, when cloning of these fragments into vectors may be required, concentration of the diluted (fragmented) DNA and removal of impurities is demanded as well.

With small adaptations to the **MagSi-DNA clean<sup>FIX</sup>** protocol, size fractionation, purification and concentration of DNA fragments is possible. It is therefore a method that can be used to circumvent time consuming, and difficult automation methods like extraction of DNA fragments from gel slices, or serial HPLC fractionation.

MagnaMedics Diagnostics BV has tested the potential use of **MagSi DNA clean<sup>FIX</sup>** kit for specific size fractionation and purification of DNA fragments, as shown in Figure 8. A two-step procedure has been developed based on selective binding of DNA fragments, enabling scientists to isolate sizes with a defined lower and upper size limit. Initially, fragments longer than needed are removed via binding to the MagSi DNA clean particles. In the second step, the fragments of selected size(s) are isolated. This simple approach can significantly increase the overall throughput when implemented on liquid handling stations.



**Figure 5:**

**Left panel:** 2 µg of 100 bp DNA ladder (BL), Kapa Biosystems Code: KK6302 ([www.Kapabiosystems.com](http://www.Kapabiosystems.com)), was size fractionated using adapted MagSi DNA clean protocols for PCR clean up. In six parallel isolations, cutoff was changed from just below 150 bp (lane 1) up to just below 1000 bp (lane 6). Lane 7 shows the original 100 bp ladder.

**Right panel:** Lanes 7 – 12 show the result of a two-step DNA fractionation approach enriching fragments of 150 - 400 bp (7), 150 - 300 bp (8), 150 - 200 bp (9), 300 - 800 bp (10), 300 - 600 bp (11), 300 - 400 bp (12). Lane BL shows the original 100 bp ladder. **Arrows** indicate 150 bp and 1000 bp (left image) and 150 bp and 800 bp (right image)



## Ordering information

Art. No	Product description
MD60013	MagSi-DNA clean <sup>FIX</sup> kit 150 purifications
MD60014	MagSi-DNA clean <sup>FIX</sup> kit kit 15.000 purifications

## MagnaMedics Diagnostics B.V.

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