

DNA Total Solution for Illumina



Published by Vazyme (688105.SH)

For Biotech Business:

♦ +86 25-83772625

- ⊠ global@vazyme.com
- Red Maple Hi-tech Industry Park, Nanjing, PRC
- ☐ Visit Our Website:
- www.vazymebiotech.com











About Vazyme

Founded in 2012, Vazyme is now a leading biotechnology company in China, conducting research and development both in technology and products focusing on functional proteins, such as enzymes, antigens, antibodies, and polymer organic materials. Relying on an in-house generic technology platform, Vazyme has successively moved into various business areas ranging from biological research, in vitro diagnosis (IVD) to biopharmaceutical. Equipped with both independent technology development and terminal products manufacturing capabilities, Vazyme is now ready to power the biotechnology industry.





Science and Technology Make a Healthier Life

DNA Total Solution for illumina

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Background

Brief Introduction

Sequencing technology can be traced back to the 1950s. In 1953, Watson and Crick revealed the double helix structure of DNA and the report of early sequencing technology appeared the following year. Sanger et al. invented the double terminal termination sequencing method in 1977, and then ABI company launched the first automatic nucleic acid sequencer on this basis. Since then, sequencing technology has entered the golden age of the first generation sequencing technology represented by the human genome project. However, first-generation sequencing technology is limited in practical application due to its low throughput. With the launch of Roches 454 sequencing platform in 2005, the sequencing industry began to entered the period of next generation sequencing.



Chromosome and DNA structure

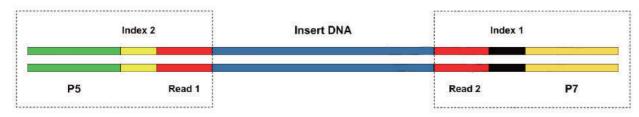
Next generation sequencing (NGS) is currently the most widely used technology and enables rapid and accurate determination of the genetic sequence of any organism, it can provide valuable information for almost all biological questions. With the development of NGS, the Illumina sequencing platform has become the mainstream sequencing technology on the market due to its high throughput and wide market acceptance. NGS usually includes the following processes:

NGS Process



DNA Library Preparation

DNA library construction is the foundation of the NGS library technology, and other library building methods have evolved on the basis of this method. Using the library structure in the figure as an example, the Insert DNA is the DNA fragment to be sequenced, the read1/read2 region is the primer complementary sequence to the DNA sequence to be tested during sequencing, the Index1/index2 is used to distinguish different library samples, and P5/P7 is able to complement the sequence on the sequencing chip to generate DNA sequencing clusters. In brief, DNA library construction is the process of adding adapter sequences for the DNA sequence to be tested.



Construction of 2-ended index library for Illumina

DNA library preparation

Feature	Product Name	Cat No.#
Conventional DNA library preparation	VAHTS Universal DNA Library Prep Kit for Illumina V3	ND607
DNA library preparation by enzymatic fragmentation	VAHTS Universal Plus DNA Library Prep Kit for Illumina	ND617
DNA library preparation by enzymatic fragmentation	VAHTS Universal Plus DNA Library Prep Kit for Illumina V2	ND627
DNA library preparation for FFPE	VAHTS Universal Pro DNA Library Prep Kit for Illumina	ND608
Multiplex amplicon DNA library preparation	VAHTS AmpSeq Library Prep Kit V3	NA210

Magnetic beads

Feature	Product Name	Cat No.#
DNA purification and sorting on magnetic beads	VAHTS DNA Clean Beads	N411

Quantification

Feature	Product Name	Cat No.#
Library absolute quantification kit	VAHTS Library Quantification Kit for Illumina	NQ101-104
Library quantification standards	DNA Standard 1-6	NQ105
Library dilution buffer	VAHTS Universal Plus DNA Library Prep Kit for Illumina V2	NQ106
dsDNA specific detection	Equalbit dsDNA/1 × dsDNA HS Assay Kit	EQ111/121

Separate Modules / Enzymes

Feature	Cat No.#
VAHTS® Universal End preparation Module for Illumina	N203
VAHTS® Universal Adapter Ligation Module for Illumina	N204
VAHTS Universal Plus Fragmentation, End Preparation & dA-Tailing Module for Illumina	N209
VAHTS HiFi Universal Amplification Mix for Illumina	N618
VAHTS AmpSeq Multi-PCR Module V3	NA215
T4 DNA Polymerase	N101
T4 Polynucleotide Kinase	N102
T4 DNA Ligase (Rapid)	N103
DNA polymerase I Klenow fragment	N104
DNA polymerase I Klenow fragment exo	N105
Phi29 MAX DNA Polymerase	N106
Phanta® Uc Super-Fidelity DNA Polymerase for Library Amplification	P507

DNA Library Preparation

Product Name	Cat No.#	Size	Feature
VAHTS Universal DNA Library Prep Kit for Illumina V3	ND607	24 rxn/96 rxn	Conventional DNA library preparation
VAHTS Universal Plus DNA Library Prep Kit for Illumina	ND617	24 rxn/96 rxn	DNA library preparation by enzymatic fragmentation
VAHTS Universal Plus DNA Library Prep Kit for Illumina V2	ND627	24 rxn/96 rxn	DNA library preparation by enzymatic fragmentation
VAHTS Universal Pro DNA Library Prep Kit for Illumina	ND608	24 rxn/96 rxn	DNA library preparation for FFPE
VAHTS AmpSeq Library Prep Kit V3	NA210	24 rxn/96 rxn	Multiplex amplicon DNA library preparation

VAHTS Universal DNA Library Prep Kit for Illumina V3

Description

VAHTS Universal DNA Library Prep Kit for Illumina V3 is rapid and efficient DNA library construction optimized for sequencing platform. Reagents are available in a mix format, which allows easy and rapid library construction, resulting in successful library construction in as little as 75 min. VAHTS Universal DNA Library Prep Kit for Illumina V3 provides a substantial boost in library conversion and amplification library output through overall improvements to the end repair module, ligation module, and library amplification module, further improving starting volume compatibility (100 pg - 4 µg) while being compatible across multiple template types (gDNA, FFPE DNA, cfDNA, Amplicons, etc.), seamless adapter targeted capture process. All reagents provided in the kit undergo rigorous quality control and functional validation that maximizes stability and reproducibility of library construction.

Features

1/ Higher molecular diversity

With the same amount of fragmented DNA as the template, the PCR free library was constructed according to the database construction process of each company, and then the qPCR library was quantified. VAHTS Universal DNA Library Prep Kit for Illumina V3 (#ND607) performs better in library conversion rate than similar products and Vazyme's previous generation products (#ND606), ensuring the richness of the library.

2/ Superior library amplification efficiency

With the same amount of fragmented DNA as the template, the library was constructed according to the data_base construction process of each company, and the library concentration was detected by Qubit. The results show that VAHTS Universal DNA Library Prep Kit for Illumina V3 (#ND607) has greatly improved the library yield compared with similar products and Vazyme's previous generation products (#ND606), which could effec_tively reduced the possibility of introducing amplification bias with high cycle number.

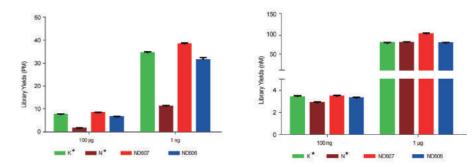


Figure 1: Comparison of ND607 with other companies' products of the similar type and ND606, effective library output.

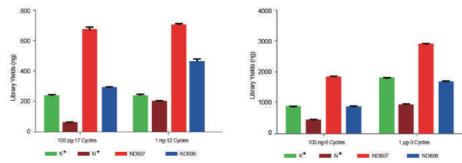


Figure 2: Comparison of ND607 with other companies' similar products and ND606 library output under different cycles.

ND607 ND608

3/ Leading sequencing data quality in the industry

Library construction was performed using 1 ng of fragmented Arabidopsis gDNA as a template and sequencing was performed using Hiseq X10 PE150 referring to the library process of each company. The results showed that the VAHTS Universal DNA Library Prep Kit for Illumina V3 (#ND607) performed better on sequencing data quality compared to K* products and N* products.

Samples	Raw_reads	Clean_reads	Clean_GC	Clean_Q30	Dup	Adapter	Mapping rate	Unique rate	Coverage
ND607	3323296	3012830	39.0%	79.0%	14.2%	13.9%	93.3%	83.3%	66.5%
ND606	3265738	2938598	39.1%	79.6%	15.1%	14.4%	91.2%	81.7%	65.0%
K*	3265760	2779182	39.0%	78.8%	15.9%	14.9%	65.9%	82.8%	46.2%
N*	4898572	3103820	39.0%	73.7%	19.2%	36.6%	84.7%	81.9%	65.5%

Table 1: Sequencing data quality analysis of ND607 and paralogs.

VAHTS Universal Pro DNA Library Prep Kit for Illumina

Description

VAHTS Universal Pro DNA Library Prep Kit for Illumina is a kind of Illumina high-throughput sequencing platform library construction kit containing DNA repair module. This kit contains DNA damage repair module, which can effectively repair DNA damage caused by formalin fixed paraffin embedding (FFPE). At the same time, it can also be compatible with ordinary DNA samples without affecting the quality of normal DNA sample library. By modifying the end repair module, ligation module and library amplification module, 100 pg - 1 μg input DNA is converted into a special library for Illumina platform, which greatly improves the conversion rate of library and the output of amplified library. It is efficient and compatible with the targeted capture process, which helps to complete the experiment of high-quality library construction.

Features

1/ Type of FFPE damage and repair ability of vazyme pppe DNA repair mixture

FFPE damage type	Deamination of cytosine to uracil	Cutting and notching	Base oxidation	3'-end blocking	DNA fragmentation	DNA-protein crosslinking
Is DNA damage repaired	Yes	Yes	Yes	Yes	No	No

2/ Efficient repair of base damage

The FFPE sample was used as a template for library construction, and His Seg X 10 PE150 was used for sequencing. The off-line data were compared with the mouse genome database on NCBI, and the number of abnormal base mutations in the sample was counted. The results showed that the efficient repair function of Vazyme ND608 significantly reduced the number of abnormal base mutations.

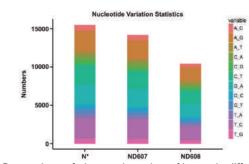


Figure 1: Comparison of abnormal number of bases in different kits.

3/ Superior library conversion

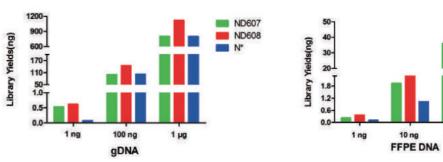


Figure 2: Comparison of transformation rates of different kit libraries. The results show that for different samples and input, ND608 has superior library conversion.

4/ Superior library amplification effects

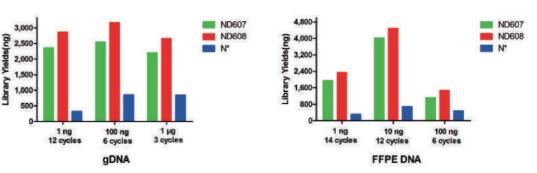


Figure 3: Comparison of amplification efficiency of different kit libraries. The results showed that ND608 had higher library amplification efficiency for different samples and input DNA. At the same yield, the number of cycles required for ND608 can be reduced by 2-3 cycles, thus reducing the amplification preference introduced by high cycle numbers.

VAHTS Universal Plus DNA Library Prep Kit for Illumina

Description

VAHTS Universal Plus DNA Library Prep Kit for Illumina is an ultra rapid DNA library construction kit that combines DNA fragmentation, end repair, and dA tailing in a single step via enzymatic reactions. This kit is extremely compatible and works with gDNA (genomic DNA) of different species' origins, such as animals, plants, and microorganisms, FFPE DNA, etc., 100 pg - 1 µg of DNA by adjusting the interruption time fragmentation of templates into target inserts. The experimental procedures are easy to perform, time-saving, and more labor-saving, especially for library construction with high-throughput samples and automated platforms. All reagents provided in the kits were subjected to rigorous quality control and functional validation, which maximized stability and reproducibility of library construction.

Features

1/ Excellent template type compatibility

The gDNA of 100 ng mouse tissue, HEK293 cells, whole blood samples (Fig. 1) and FFPE samples of different quality levels (Fig. 2) were input respectively, and the fragmentation time was adjusted to construct the library. The results showed that the distribution range of DNA template libraries for different types was basically the same, indicating that Vazyme #ND617 kit had wide compatibility of template types. At the same time, the kit had strong compatibility with FFPE samples of different quality levels.

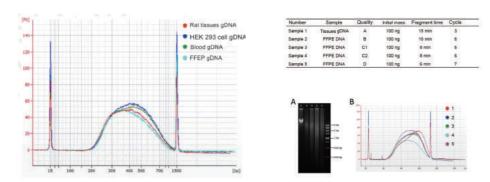


Figure 1: Fragment size distribution of libraries constructed with different types of DNA templates. Figure 2: Fragment size distribution of libraries constructed from FFPE DNA of different quality grades.

2/ Broad input volume compatibility

The library was constructed with different initial amounts of salmon sperm gDNA as templates, and the fragmentation conditions were 37°C and 22 min. The results showed that the distribution range of the library was basically the same for different initial amounts of the same species and the same fragmentation time, indicating that the input amount of the kit had a wide compatible range.

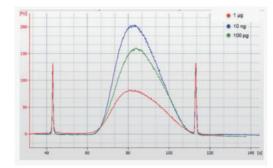


Figure 3: Fragment size distribution of libraries constructed with different DNA starting amounts.

3/ Excellent library conversion

100 pg, 10 ng, 1 µg gDNA and 50 ng FFPE DNA were used as templates, and the PCR free library was constructed according to the operation process of fragment enzyme DNA library construction kit of different manufacturers. The library was quantified by qPCR and the library conversion rate was converted. The results showed that Vazyme #ND617 had a higher library conversion rate than the same type of products of other companies.

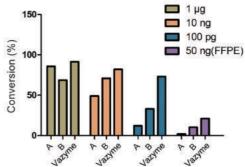


Figure 4: Comparison of conversion rate of each product library.

4/ High quality sequencing data

1 ng of Arabidopsis gDNA was used to construct PCR amplification library, and sorted according to the 350 bp insertion fragment. In the later stage, Hiseq X10 PE 150 was used for sequencing. The results showed that Vazyme #ND617 was superior to similar products of other companies in terms of sequencing data quality.

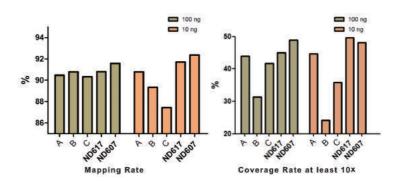


Figure 5: Comparison of sequencing data quality.

VAHTS Universal Plus DNA Library Prep Kit for Illumina V2



Description

VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 (Vazyme #ND627) specifically optimized and upgraded the system on the basis of ND617 to reduce the false-positive mutation in the construction of library by fragment enzyme method from the source. Matching with double ended UDI UMI adapters can reduce false positive mutations and make the detection results more accurate and reliable.

Features

1/ High library conversion

The PCR free library was constructed with salmon genomic DNA as template, and the input amount was 1 ng and 1 µg. Compared with similar products of other companies, Vazyme #ND627 had basically the same library conversion rate.

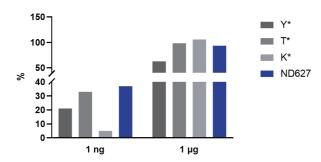


Figure 1: Library transformation rate under different input.

2/ Low background noise

Analyze the proportion of artifact reads of the tested samples. After the library is constructed with Vazyme #ND627, the proportion of artifact reads could be as low as 0.1%, which greatly reduced the false positive detection of SNV/Indel.

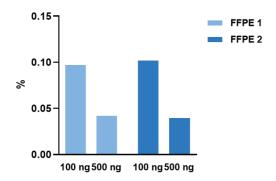


Figure 2: Test to background noise ratio analysis.

3/ Low SNP and indel calls

The sensitivity and accuracy of comprehensive SNP and indel detection were F-measure scored, and Vazyme #ND627 out performed other company products slightly in SNP and indel detection.

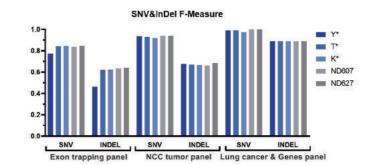


Figure 3: Comparison of conversion rate of each product library.

VAHTS AmpSeq Library Prep Kit V3

Description

VAHTS AmpSeq Library Prep Kit V3 is an amplicon library building kit based on ultra multiplex PCR, introducing core technologies such as terminal primer digestion and connecting adapters to form a library. On the basis of the previous generation (Vazyme #NA201), this kit improves the coverage and uniformity degree of the library. The upgraded digestion module can effectively solve the pollution risk of PCR products and improve the utilization of data, making the results more stable and reliable, and helping researchers and testers to build the library simply, quickly and with high quality.

Features

1/ High output

The process is simple with as little as 5 h from DNA to library amplification, less than 1.5 h by hand, no purification steps in between, and easy automation.

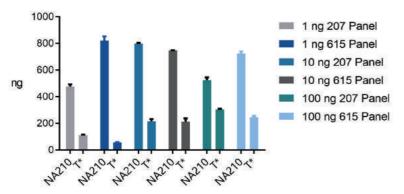


Figure 1: Library yield under different inputs.

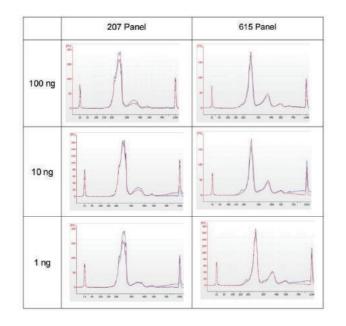


Figure 2: Comparison of peak patterns of DNA library under different inputs.

2/ Better coverage and uniformity degree

Under different starting amounts and panel conditions, the DNA library constructed with 293T cell genomic DNA had better coverage and uniformity than similar products of T* company.

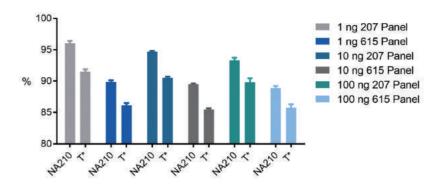


Figure 3: Coverage of genomic DNA from 293T cells.

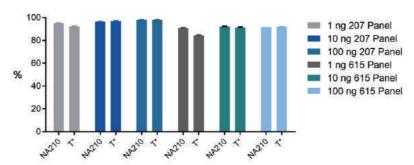


Figure 4: The DNA uniform of 293T cells depth over 2%.



VAHTS DNA Clean Beads

Product Name	Cat No.# Size		Feature	
VAHTS DNA Clean Beads	N411	5 ml/60 ml/450 ml	DNA purification and sorting on magnetic beads	

DNA Total Solution for Illumina

VAHTS DNA Clean Beads

VAHTS DNA Clean Beads

Description

VAHTS DNA Clean Beads are based on the principle of SPRI (Solid Phase Reverse Immobilization), and are suitable for DNA purification and size selection for the high-throughput sequencing libraries construction. VAHTS DNA Clean Beads are compatible with DNA and RNA library building kits of various brands and library building processes reported in the literature. They are used in exactly the same way as AMPure XP Beads which are widely used at present. The production and size distribution of the library are consistent with the height of AMPure XP Beads. Therefore, AMPure XP Beads can be seamlessly replaced to effectively reduce the cost of library construction.

Mechanism

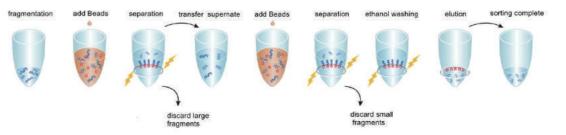
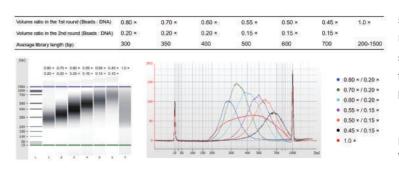


Figure 1: The operation steps of magnetic bead sorting DNA fragments.

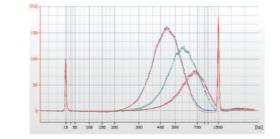


50 ng human DNA template was used to construct DNA library. Using VAHTS DNA Clean Beads and AMPure XP Beads to select the average length of about 470, 570, 670 bp library in the same conditions (corresponding insert size is 350, 450, 550 bp). Using the Agilent 2100 Bioanalyzer to analyze.

Figure 2: Schematic diagram of analyzing the sorted library with Agilent 2100 Bioanalyzer.

DNA Library Construction, Compared With AMPure XP Beads

50 ng human DNA template was used to construct DNA library. Using VAHTS DNA Clean Beads and AMPure XP Beads to select the average length of about 470, 570, 670 bp library in the same conditions (corresponding insert size is 350, 450, 550 bp). Using the Agilent 2100 Bioanalyzer to analyze.



- VAHTS[™] DNA Clean Beads 470 bp
- AMPure XP Beads 470 bp
- VAHTS™ DNA Clean Beads 570 bp
- AMPure XP Beads 570 bp
- VAHTS™ DNA Clean Beads 670 bp
- AMPure XP Beads 670 bp

Figure 3: Using Agilent 2100 Bioanalyzer to analyze DNA library.

Quantification

Product Name	Cat No.#	Size	Feature
VAHTS Library Quantification Kit for Illumina	NQ101-104	500 rxn each	Library Absolute Quantification Kit
DNA Standard 1-6	NQ105	8 rxn	Library quantification standards
Library Dilution Buffer	NQ106	50 ml	Library Dilution Buffer
Equalbit dsDNA/1 × dsDNA HS Assay Kit	EQ111/121	100/500 assays	dsDNA specific detection

VAHTS Library Quantification Kit for Illumina

Description

This product is a special kit for Illumina platform high throughput sequencing library concentration determina □tion using dye qPCR method. Its working principle is to draw a standard curve using standard materials, and then calculate the absolute concentration of the library to be measured according to the standard curve. The kit uses VAHTS SYBR qPCR Master Mix (a novel dye qPCR premix based on antibody method heat start). The premix has many advantages such as high specificity, high amplification efficiency, wide GC content adaptability and high detection sensitivity, which makes it suitable for the absolute quantification of library.

Features

1/ High amplification efficiency

The Step-One-Plus platform was used to test VAHTS Library Quantification Kit for Illumina and similar products of K* company. The results showed that the standard curve equation R2 drawn by Vazyme library quantitative standard and K* company's library quantitative standard were 1 and 0.9999, respectively, and the ampli□fication efficiency was 95% and 94%, respectively.

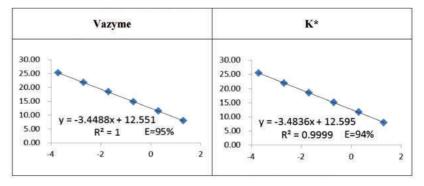


Figure 1: Comparison of standard curves.

2/ Accurate quantitative results

The quantitative results of Vazyme NQ series and K* company's similar product libraries were basically the same for different lengths of library.

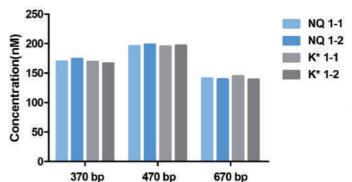


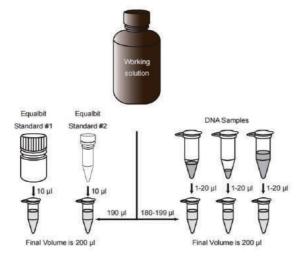
Figure 2: Comparison of quantitative results of different length Libraries.

Equalbit 1 × dsDNA HS Assay Kit

Description

Equalbit 1 × dsDNA HS (High Sensitivity) Assay Kit is a simple, sensitive and accurate fluorescence quantita tive assay kit for double stranded DNA (dsDNA). Equalbit 1 × dsDNA HS Assay Kit includes premixed working solution (containing fluorescent dye) and dsDNA standard. The kit has excellent linear relationship with dsDNA samples in the range of 0.2 - 100 ng, and can accurately quantify samples from 10 pg/µl to 100 ng/µl. For some conventional contaminants, such as RNA, salt, free nucleotides, proteins, solvents, detergents and so on have excellent tolerance.

Mechanism



Features

1/ Simple Operation

During the experimental operation, there is no need to prepare working solution. The premix is directly divided into 0.5 ml tubes to add standard or test samples, and the data could be readed by using Qubit Fluorometer 2.0, 3.0, 4.0.

2/ High Sensitivity

For 12 dsDNA samples with different concentrations, Vazyme #EQ121 and similar products of T* company were used for linear determination, and the fluorescence value was read by Qubit Fluorometer 3.0. The results showed that the total amount of dsDNA samples had a good linear relationship in the range of 0.2 - 100 ng.

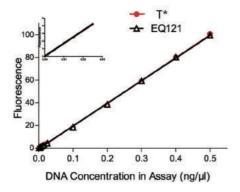


Figure 1: Comparison of 0.2-100 ng linear relationship

The results showed that the deviation rate between Vazyme #EQ121 and similar products of T* company (difference between Vazyme #EQ121 and similar products of T* company/measured value of similar products of T* company) was within 10% near the critical test point.

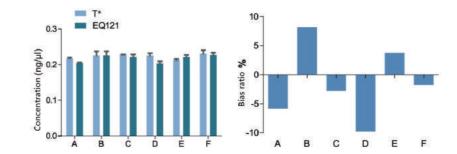


Figure 2: Plot of results for different operators testing low concentration as well as bias rate

3/ Exceptional Specificity

The results showed that Equalbit 1 × dsDNA HS Assay Kit could specifically bind dsDNA, and even in the presence of RNA, it could still accurately quantify dsDNA, and its performance was comparable to that of similar products of T* company.

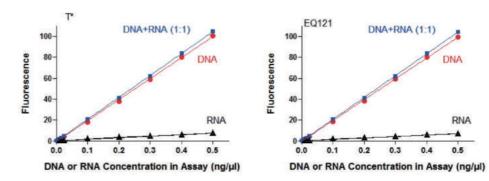


Figure 3: dsDNA specificity detection diagram of EQ121 and similar products of T* company.

4/ Dyes Bind Quickly

The results showed that the deviation between the test results of the two kit after adding samples for 5 min and 1 min was within 10%, indicating that the binding speed of the two kit to dsDNA samples was the same, and saturation could be achieved within 2 min.

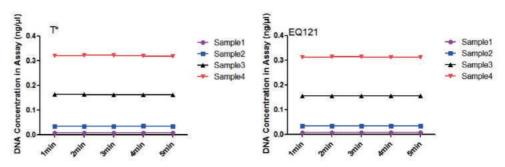


Figure 4: Binding speed of EQ121 with dsDNA dye of similar products of T* company