

Certificate of Analysis

BDT V3.5 Reagent storage standard	Code: BS-021
	Dept: QC-D
Shanghai Na Yuan Technology Co., Ltd. (Controlled Document)	

1 . Purpose:

Specify and define the standard operating production process for BDT V3.5 sequencing reagents to be stored

2. Scope:

Suitable for production and quality control personnel

3. Responsibility and authority:

Production and operation personnel must strictly follow this procedure; If it is found that this procedure is not applicable, it is necessary to put forward a more suitable plan to the group leader and the department manager in time, modify it according to the Document Control Procedure, and form a new standard process after review and approval.

4. Operation procedure:

4.1 Reagent appearance and packaging

Packaging using nalgene 30ml standard reagent packaging. Dispense in nitrogen station. The packing size is weighing and sub-packing, $21.5g \pm 2\%$ per bottle.

4.2 Sequencing test

4.2.1 Using standard plasmid tests:

Sequencing reaction system:

Reagent	10 μ L reaction system
H ₂ O	5.7 μ L
-21 M13 Control Primers (3.2 μ M)	1 μ L
BDT Mix	0.5 μ L
5x Sequencing Reaction Buffer	1.8 μ L
pGEM-3Zf(+)Control (200ng/ μ L)	1 μ L
Total volume	10 μ L

Reaction conditions:

Parameter	Stage/step				
	Incubate	35 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	1:00	00:10	00:05	4:00	Hold until ready to purify.

Test result achieved:

Initial signal	Length read	900bp signal
3000-6000	1000bp	500-1500

4.2.2 Using standard PCR product tests:
Sequencing reaction system:

Reagent	10µL reaction system
H ₂ O	5.8µL
BST-OL-1 Control Primers (5µM)	1µL
BDT Mix	0.3µL
5x Sequencing Reaction Buffer	1.9µL
BST-STD2 PCR product (10ng/µL)	1µL
Total volume	10µL

Reaction conditions:

Parameter	Stage/step				
	Incubate	30 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	1:00	00:10	00:05	4:00	Hold until ready to purify.

Test result achieved:

Initial signal	Length read	650bp signal
4000-8000	650bp	1500-2500

4.2.3 Test using a gene fragment with a certain structure (plasmid):
Sequencing reaction system:

Reagent	10 μ L reaction system
H ₂ O	5.7 μ L
BST-OL-1 Control Primers (3.2 μ M)	1 μ L
BDT Mix	0.5 μ L
5x Sequencing Reaction Buffer	1.8 μ L
BST-Plasmid1 Control (200ng/ μ L)	1 μ L
Total volume	10 μ L

Reaction conditions:

Parameter	Stage/step				
	Incubate	35 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	1:00	00:10	00:05	4:00	Hold until ready to purify.

Test result achieved:

Initial signal	Length read	800bp signal
4000-8000	900bp	500-1500

4.2.4 G peak adjustment test:
Sequencing reaction system:

Reagent	10 μ L reaction system
H ₂ O	5.7 μ L
M13F Control Primers (3.2 μ M)	1 μ L
BDT Mix	0.5 μ L
5x Sequencing Reaction Buffer	1.8 μ L
BST-STD1 Control template (200ng/ μ L)	1 μ L
Total volume	10 μ L

Reaction conditions:

Parameter	Stage/step				
	Incubate	35 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	1:00	00:10	00:05	4:00	Hold until ready to purify.

Test result achieved:

The G peak signal is more than 50% higher than the original version.

Quality Inspection Report

Product No.: **BDT-100** Lot No.: **20240923**
 Date of Manufacture: **23SEP2024** Expiration Date: **22SEP2025**

Appearance and sequencing ability test results:

Detection entry	Detection result	verify
Quantity in storage	600ML	√
Appearance verification	Pink transparent	√
Standard plasmid test results	pass	√
Standard PCR product test results	pass	√
Structural fragment detection results	pass	√
G-peak signal	2500	√

Standard plasmid test results:

No.	Samples	Initial signal	Length read	900bp signal
1	Sample	3000-6000	1000bp	500-1500
2	BDT V3.5	5000	900bp	1000

Standard PCR product test results:

No.	Samples	Initial signal	Length read	900bp signal
1	Sample	3000-6000	1000bp	500-1500
2	BDT V3.5	5500	850bp	900

Structural fragment detection results

No.	Samples	Initial signal	Length read	900bp signal
1	Sample	3000-6000	1000bp	500-1500
2	BDT V3.5	5000	800bp	600

Viscosity test result:

No.	Samples	Rotational speed	Pass state	viscosity
1	Sample	150	Uniform velocity	3.3
2	POP-7	150	Uniform velocity	3.3