

(54) - H5 A

RNA (RT-PCR) 가 H5
 H5 A B C H5
 H5 가 H5
 1

H5 A
 가 (avian ; A)
 (lineages) A (phylogenetic)
 (transmission)
 (aquatic bird)가

) 1889 A 가 (Serological) (virological)
 가 6 HA (Subtype) 가
 00 H3 , 1918 H1 3 가 HA - 1889 H2 , 19
 1 H5N1 가 A , 1957 H2 , 1968 H3 , 1977 H
 3 H5 , H5 1997

(RT-PCR)
 가 가,
 H5 A (immunodiagnostic assay)
 (haemagglutinin inhibition, HI)
 가 가,
 (antigenicity) (cross
 - reactivity)

가

H5

H5

A

H5

A

H5

A

H5

A

H5

RNA

;

:

H5

RNA

;

;

- H5

A

RNA

1 DNA

;

- RNA
DNA

DNA

2 DNA

DNA

DNA

1 DNA
DNA

가 H5

RNA

- H5

RNA

DNA

;

- RNA

DNA

DNA

DNA

- -

H5

A

RNA

1 DNA

;

- DNA

DNA

2 DNA

DNA

DNA

DNA
DNA

가 -

H5

DNA

RNA

DNA

- H5 A RNA DNA ;

- DNA DNA DNA .

SEQ ID NO: 5, 6, 7, 9, 10 DNA 11 DNA DNA 1 DNA

- H5 A RNA 1 DN
A H5 A RNA

- (immobilizer) DNA DNA DNA ,

- H5 A RNA 1 DN
A H5 A RNA

- (signal generator) DNA DNA DNA
가

1 2 DNA H5 A

- H5 RNA ;

- 1 2 DNA , ,

H5 RNA ;

-

가 (Nucleic acid sequence-based amplifi
cation, NASBA) RNA . NASBA 가 , 가 , H5 H5

A RNA 가 (RNA) . H5 (hae
magglutinin) 1756 , 5'

1 가 RNA H5 H5 1 가 , , lymphocytes, PBMC/PBL), abs), (cerebrospinal fluids), (peripheral blood mononuclear cells/peripheral blood (throat swabs), (dermal lesion sw (cervical smears), (food matrices), 가

H5 RNA H5 RNA , NASBA RNA

(lysis agent) RNA

가, RNA 48

2 , -70 RNA

5M (guanidine thiocyanate) Tris/HCl

3 (10) ent) 가 (10) , / (14) (12) (adsorb % , , (12) , 5M (eluent) Tris/HCl , Tris/HCL , 70 (14)

(12) H5 RNA A C (amplification agent) , , NASBA DNA

4 , , NASBA H5 RNA RNA RNA RNA (annealing) T7 RNA A (22) A (22) -가 RNA H5 RNA RNA (20) 가 , RNA , A

H5 DNA H5

A (22) H5 RNA (20) DNA H5

1107 1132 RNA (20) H5

1107 1132 A 6 SEQ ID NO: 1 H5 DNA

SEQ ID NO: 1 ' (back)' 5'-3' ' (front)'

1040 H5 1060 1140 (SEQ ID NO: 2, 7) 1160 (SEQ ID NO: 3, 8) A (22)

RNA NO: 4 (9) RNA DNA A (22) 가 5' RNA T7 RNA DNA SEQ ID A가 H5

A가 H5 RNA A A 3' (Avian Myoblastosis) (AMV-RT) A (24)가 :

(a) H5 RNA DNA ;

(b) RNA DNA .

DNA:RNA (26) H5 RNA RNase H A (22) B (28)가 A (24) B (28) A (24) B (28) H5 B (28) H5 B (28) H5 B (28) AMV-RT H5 RNA

28) 1 DNA H5 H5 914 940 (SEQ ID NO: 5, 10) 866 961 (SEQ ID NO: 6, 11) 846 981 (SEQ ID NO: 7, 12)

A B 1 DNA B 2 DNA 가 DN , SEQ ID NO: 8 (13) DNA 2 DNA B 5' 2 DNA B , SEQ ID NO: 8

A (32) B (28) A (24) T7 RNA (30) RNA H5 RNA T7 RNA T7 RNA RNA RN

H5 A B C B C DNA H5 DNA : B C DNA H5

- H5 RNA 1 DNA ;

- DNA DNA 2 DNA B , 2 D NA

C H5 B

(SEQ ID NO: 9, 14) C 1 DNA H5 1017 1042 (SEQ ID NO: 10, 15) H5 950 1083 (SEQ ID NO: 11, 16) 970 1063

C A (22) C A (22) H5 RNA B (28) H5 RNA (32)가 RNA A (22) B (28) H5 RNA :

(a) H5 RNA () RNA ;

(b) B C가 2 DNA , RNA .

RNA (32) T7 RNA RNA 가

T7 RNA RNA

RNA (32) 5 RNA (32) , (40) ,

(41) 5 (41)

(ruthenium-bipyridine complex, $[Ru(bpy)_3]^{2+}$) (41)

(, ^{32}P), (/), (), (el

electrochemiluminescence molecules)

B C가 DNA 2 DNA RNA (32) 가

B C가 H5 1 DNA

가 : RNA (32) H5 RNA ;

RNA (32) A, B, C, , 가

(capture probe, 42) RNA (32) RNA (32)

(42) H5 RNA (32) RNA 가

(42) (immobilizer, 44) RNA (32)

2) working electrode) (magnetic) 5 (44) RNA (32) (w

(42)가

A B A C RNA

RNA

5 가 RNA (40) , 가 (42)

(42) 가 (40) 가 가 (42) 가 ,

A, B, C 가 DNA DNA

A, B, C

1 H5 A

2 H5 A
 3 RNA
 4 DNA , A B H5 A RNA
 5 RNA 가 RNA
 6 19 DNA SEQ ID NO: 1 14 ,

A.

50 x 0.9 ml (5M , X-100, Tris/HCl)

B.

5 x 22 ml (5M , Tris/HCl)
 5 x 0.8 ml (-)
 5 x 1.5 ml (Tris/HCl)

C.

5 x 60 µl (T7 RNA , -
 (AMV-RT), RNase-H)
 5 x 10 mg (Reagent spheres) (, (dithiothreitol) MgCl2
).
 1 x 0.6 ml (Tris-HCl, 45% DMSO)
 1 x 1.6 ml KC1
 1 x 70 µl H5-

D.

1 x 0.9 ml ECL (- DNA : 5 g/L 2-
)
 1 x 0.7 ml H5- ((Biotinylated)- : 5 g/L 2-
)
 2 x 1.7 ml (Instrument Reference Solution, -)

가 :

70% (v/v) (96-100% (v/v), ACS);	-	Merck 1.0098 3
,		SIGMA A4206
RNase-		SIGMA D5758

A. _____

37 30
10

B. _____

: 10 , 2 -20

1.

37 30
10

C. _____

: -20 -70 가 2

1. /KCl

80 μ l 가

KCl 30 μ l 가

2. RNA-

/KCl 110 $\mu\ell$ H5- 10 $\mu\ell$ 가 .

3.

가 가 .

D. _____

2 8

1.

amplicons) H5- RNA (

2. H5 RNA (hybridisation solution)

H5-

N H5 RNA- :

H5 RNA- (N+2) x 10 $\mu\ell$ 가

ECL (N+2) x 10 $\mu\ell$ 가

_____ (*in vitro*) RNA _____

A.

1. 30 .

2. 10,000 x g 30 .

3. RNA 100 $\mu\ell$ 가 .

:

-70

2 8 14

25 48

4. RNA/ 50 $\mu\ell$ 가 .

5. RNA/ / 10 (2

).

6. RNA/ / 10,000 x g 30 .

7. () 1 $\mu\ell$ 가 .

8.

9. 10,000 x g 30 .

10. (7) (9)

70 %

II. , 100 μ l

12. 56 10 가 .

13. , 50 μ l 가 .

14. .

15. 56 10 (5).

16. 10,000 x g 2 .

17. 5 μ l , 1 .

B.

1. H5 RNA , 5 μ l .

2. H5 RNA - B 10 μ l 가 . - H5 A

3. 가 65 5 .

4. 가 41 5 .

5. 5 μ l 가 가 가 .

6. 10 41 .

7. 41 90 .

8. , -20 1 .

9. - H5 A C 가 , 2 8 H5

C.

1. RNA 20 μ l 가 .

2. :

H5 RNA 5 μ l 가.

- 3.
 - 4. 41 30
 - 5. 300 $\mu\ell$ 가 가
- H5
(photomultiplier tube) 가

H5 alyzer) DNA (Perkin Elmer ABI 310 Genetic An

[1]

A B		
H5		
/ no.	H5	
	가	
258/97	4229	
977/97-2	6961	
1000/97	33835	
1258/97-2	2500	
1258/97-3	2400	
1258/97-4	10494	
1258/97-5	3089	
1258/97-9	4883	
1258/97-10		
437/9-4	5165	
437/99-6	22200	
37/99-8	5142	
437/99-10	511	
1	1	
2	1	
3	35	

[2]

A C		
H5		
/ no.	H5	
	가 (x 10 ³)	
258/97	11800	
977/97-2	5300	
1000/97	23100	
1258/97-2	61800	
1258/97-3	85100	

- 1. H5 RNA ;
- H5 RNA ;
- H5 A ;
- 2. H5 A 가 , -
- 3. 가 RNA , - H5 A
- 4. 1 DNA , 1 DNA
가
- H5 RNA 1 DNA ;
- RNA 가 H5 RNA DNA 2 DNA , DNA 1 DNA
1 DNA 가
- H5 RNA DNA ;
- RNA H5 DNA A DNA DNA -
- 5. 4 , 1 DNA SEQ ID NO: 1, 2, 3 DNA ,
- H5 A
- 6. 4 , RNA 가 T7 RNA H5 , A 2 DNA SEQ ID NO:
4 DNA , -
- 7. 4 , 2 DNA , 2 DNA ,
- H5 RNA 1 DNA ;
- DNA 2 DNA ,
- DNA 2 DNA 가 - H5 RNA 2 DNA DNA
.
- - H5 RNA DNA ;

- H5 DNA A DNA DNA , -

8.
7 ,

- H5 가 - ;

- 1 DNA SEQ ID NO: 5, 6, 7 DNA , -
H5 A .

9.
7 ,

- H5 가 ;

- 1 DNA SEQ ID NO: 9, 10, 11 DNA , -
H5 A .

10.
7 9 H5 , A 2 DNA SEQ ID NO: 8 DNA , -

11.
4 , SEQ ID NO: 5, 6, 7, 9, 10 11 DNA H5
1 DNA A 2 DNA , -

12.
1 , ,
H5 RNA 1 DNA ;
NA (immobilizer) 가 DNA 가 H5 , A D

13.
12 , 1 DNA SEQ ID NO: 12, 13, 14 DNA , -
H5 A .

14.
12 , 가
H5 RNA 1 DNA ;
 ,
A , DNA 가 , - H5

15.
- H5 A RNA 1 DNA ;
- RNA DNA DNA , DNA 1 DNA 가 H5 2 DNA RNA DNA
DNA DNA DNA 가

- H5 RNA DNA ;
- RNA DNA DNA DNA D
NA DNA .
- 16.**
15 , 1 DNA SEQ ID NO: 1, 2, 3 DNA ,
DNA DNA .
- 17.**
15 , RNA 가 T7 RNA , 2 DNA 가 SEQ ID NO:
4 DNA , DNA DNA .
- 18.**
- - H5 A RNA 1 DNA ;
- DNA DNA 2 DNA DNA D
NA , DNA , DNA 가 - DNA H5 RNA DNA
- - H5 A RNA DNA ;
- DNA DNA DNA DNA , DNA
DNA .
- 19.**
18 ,
- H5 가 - ;
- 1 DNA SEQ ID NO: 5, 6, 7 DNA , D
NA DNA .
- 20.**
18 ,
- H5 가 ;
- 1 DNA SEQ ID NO: 9, 10 11 DNA ,
DNA DNA .
- 21.**
18 20 DNA , 2 DNA SEQ ID NO: 8 DNA
, DNA .
- 22.**
SEQ ID NO: 5, 6, 7, 9, 10 11 DNA 1 DNA
DNA DNA .
- 23.**
- H5 H5 A RNA RNA 1 DNA ;
H5 A RNA
- ,

DNA . DNA , DNA

24.
 23 , DNA 1 DNA SEQ ID NO:12, 13 14 DNA
 , DNA DNA .

25.
 - H5 H5 A RNA RNA 1 DNA ;
 - H5 A RNA RNA ;
 - 가 DNA DNA DNA . ,

26.
 - H5 RNA 가 ;
 - 가 1 2 DNA , ,
 :
 H5 RNA ;
 ;
 - A 1 2 DNA H5

27.
 26 , H5 H5 RNA
 1 2 DNA . H5 A

28.
 26 , 가 RNA , H5 A
 1 2 DNA .

29.
 26 , 1 DNA 가
 - H5 RNA 1 DNA ;
 - RNA DNA 2 DNA ,
 1 DNA 가 1 DNA DNA 가 H5 RNA ,
 - H5 RNA DNA ;
 - RNA H5 DNA DNA DNA DNA 1 2 DNA

30.
 29 , H5 1 DNA SEQ ID NO: 1, 2, 3 DNA
 H5 A 1 2

DNA

31.

29 30
 SEQ ID NO: 4 DNA RNA 가 T7 RNA H5 A 2 DNA
 1 2 DNA

32.

26 ,
 - - H5 RNA 1 DNA ;
 - DNA 2 DNA 2 DNA
 ,
 DNA 2 DNA 가 - H5 RNA DNA 가 DNA
 DNA
 - - H5 RNA DNA ;
 - H5 DNA DNA DNA 1 2 DNA
 .

33.

32 ,
 - H5 가 - ;
 - 1 DNA SEQ ID NO: 5, 6, 7 DNA H5 A 1 2 DNA
 .

34.

32 ,
 - H5 가 ;
 - 1 DNA SEQ ID NO: 9, 10, 11 DNA H5 A 1 2 DNA
 .

35.

32 34 , 2 DNA SEQ ID NO: 8 DNA 1
 , 2 DNA H5 A

36.

26 ,
 A 1 DNA SEQ ID NO: 5, 6, 7, 9, 10 11 DNA H5
 A 2 DNA 1 2 DNA .

37.

26 ,
 H5 RNA 1 DNA ;

DNA 가 ,
H5 가 A DNA 가 1 2 DNA .
38.
37 , 1 DNA SEQ ID NO: 12, 13 14 DNA ,
DNA H5 A 1 2 ,
39.
26 , 가
H5 RNA 1 DNA ;
가 A DNA 가 1 2 DNA . H
40.
36 , 가, 34 2 DNA 2 DNA ,
DNA 33 H5 A 2 DNA 1 2 ,

1

과정

1. 핵산 추출



2. RNA 증폭



3. ECL 검출

2

샘플 세부 사항

- 용균 버퍼 모듈



샘플링 장소

용균 버퍼 내 수송

RAN 안정성:

- 15-30°C, 48 시간
- 2-8°C, 14 일
- -70°C, 장기간-



- 분리 모듈
- 증폭 모듈
- 검출 모듈

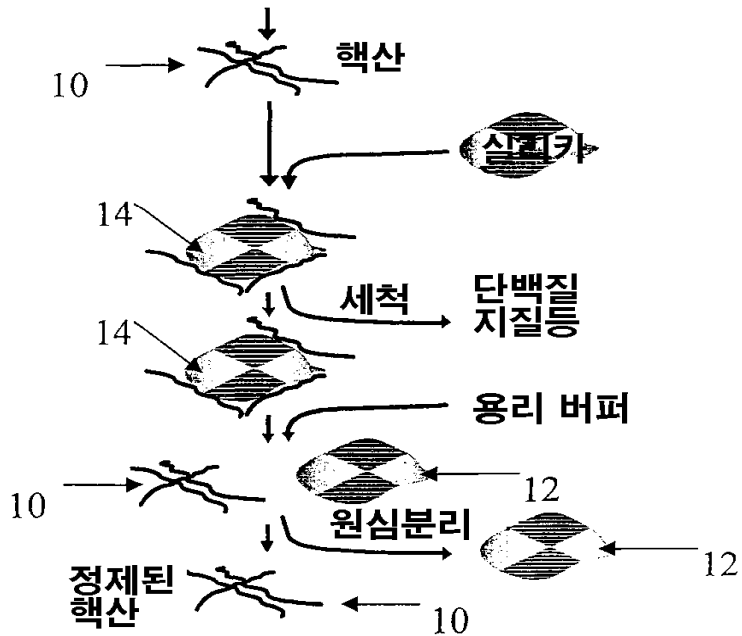


테스트 장소

3

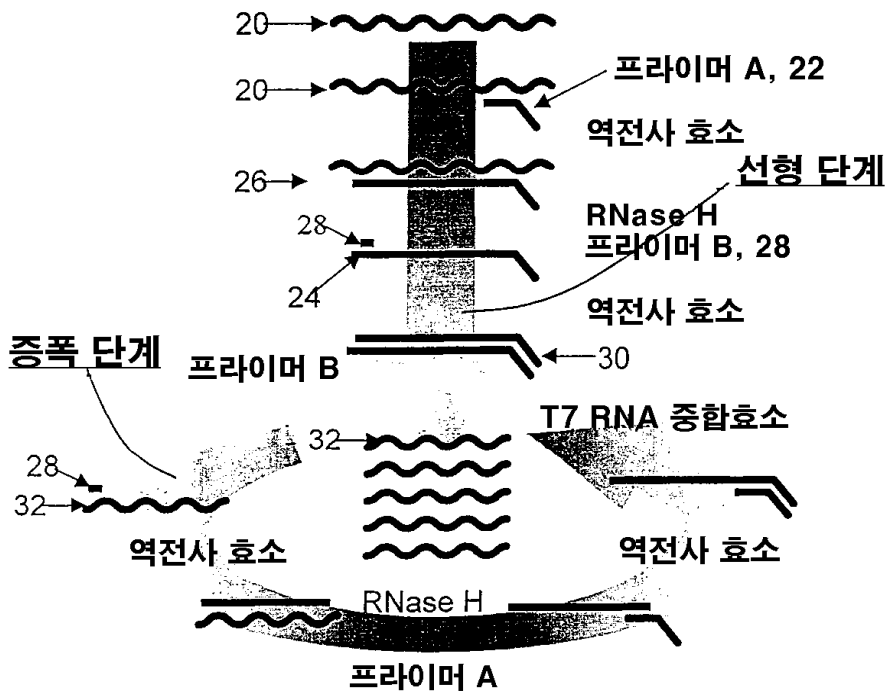
분리 : 개략적 다이어그램

- 샘플 + 용균 버퍼



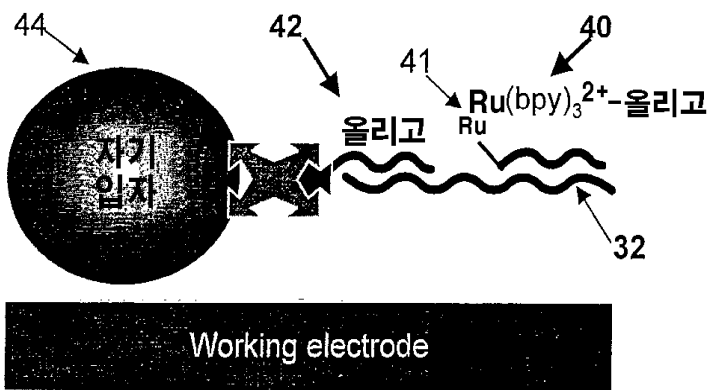
4

증폭 : 개략적 다이어그램



5

ECL 검출 : 개략적 다이어그램



- <110> Hong Kong DNA Chips Limited
- <120> A Kit for Detecting Non-Pathogenic or Pathogenic Influenza A Subtype H5 Virus
- <130> 9852618
- <140> PCT/CN01/xxxxx
- <141> 2001-09-27

<150> 00106310.0
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 atggttgta t 131