



choline .1  
 PD 50% .13  
 L-tyrosine tyrosine hydroxylase (TH, tyrosine 3-monoxygenase, EC 1.14.16.2) L-dopa(L-3,4-dihydroxyphenylalanine)  
 L-dopa aromatic amino acid decarboxylase(AADC) norepinephrine epinephrine .14  
 TH 가 tetrahydrobiopterin(BH<sub>4</sub>) tyrosine, L-dopa, catecholamine, 가 .15  
 가 TH .16 TH TH  
 가 (autoxidation) semiquinone, quinone, zwitterionic 5,6-dihydroxyindole, melanin .17,18  
 DNA,  
 TH knock-out transgenic mouse TH<sup>-/-</sup> 95% .19,20  
 TH 5  
 TH SDS-PAGE 60 kDa N-(regulatory domain) C-(catalytic domain) serine/threonine(amino acid # 8, 19, 31, 40) kinase .15,21  
 Ser 8 kinase(cdc2/cyclin A) TH  
 Ser 19 calmodulin dependent protein kinase (CaM-PK )<sup>22</sup> MAP-kinase-activated protein kinase(MAPKAP kinase) .23  
 Ser 31 protein kinase C(PKC) mitogen-activated protein kinase 1(MAPK 1) MAP kinase 2<sup>24</sup>  
 PC12 PKC

phobol ester Ser 31 가 .25-27  
 2 PKC TH  
 , PKC MAP kinase TH  
 catecholamine "cross-talk"  
 가 Ser 40 kinase(PKA, PKC, CaM-PKII) .  
 kinase (BH<sub>4</sub>)  
 PKA Ser 40 catecholamine (K<sub>m</sub>)  
 (K<sub>i</sub>) .28,29 ser 40 PKC  
 CaM-PKII .30  
 L-dopa 30 가  
 on-off wearing-off ,  
 L-dopa .31 L-dopa  
 PD L-dopa  
 가  
 가 tyrosine hydroxylase(TH),<sup>32</sup> TH BH<sub>4</sub>  
 GTPCH I,<sup>33</sup> L-dopa AADC,<sup>34</sup> brain-derived neurotrophic factor(BDNF)<sup>35</sup>  
 TH GTPCH I TH 60 가  
 TH 10 TH tyrosine hydroxylase Parkinsonian  
 PD  
 TH PD

1. retroviral vector  
 type 2 tyrosine hydroxylase(hTH63)  
 Bst XI Klenow blunt end  
 Hind Vector  
 pLNCX<sup>36</sup> Cla Klenow  
 blunt end ethanol Hind  
 insert 6.9 kb vector  
 1.7 kb  
 Qiaex kit DNA  
 DNA 90/ng insert 207/ng  
 vector가 가  
 DNA 가 2/unit T4 ligase 14  
 colony  
 alkaline lysis miniprep plasmid DNA  
 GTPCHI<sup>37</sup> Bam HI Hinc vector p  
 gHC Cla Klenow blunt  
 end Bgl  
 colony LB  
 alkaline lysis miniprep plas-  
 mid DNA

2. Transfection 1 × 10<sup>6</sup> BOSC 23  
 (virus producing cell line) 10/cm  
 12/μg DNA 30.5/μl 2 M CaCl<sub>2</sub>  
 가 0.25/M(250/μl)  
 2X HEPES DNA 30  
 retrovirus packaging cell  
 line BOSC 23 37 4  
 PBS 37 CO<sub>2</sub>  
 0.45/μm  
 -80

3. 1 × 10<sup>5</sup>/plate  
 가 1 ml polybrene(4 μg/ml)  
 PBS  
 trypsin 1/250,  
 1/1000, 1/3000 가  
 2 2-3 subcloning ring  
 single colony 6-well plate

4. TH  
 5 × 10<sup>6</sup> 300 μl lysis buffer(10/mM  
 potassium phosphate, pH 6.2, 0.2% Triton × 100)

10 sonicate 12,000/×g 15  
 4 assay  
 BSA standard  
 Bio-Rad protein  
 assay kit(BIO RAD)  
 5/μCi L-[3,5-  
<sup>3</sup>H] tyrosine 가 30  
 tyrosine 50/μl  
 5/mM DTT, 1/mM D,L-6-methyl-5,6,7,8-  
 tetrahydropterine(6-MPH<sub>4</sub>), 165/μM tyrosine,  
 50mM 2-[N-morpholino]ethanesulfonic acid  
 (MES), 3000 units/ml catalase 가  
 50/μl 50/μl 37  
 20 1/N HCl 7.5% charcol  
 suspension 1/ml . Reac-  
 tion/charcoal mixture vortex  
 hydroxylation <sup>3</sup>H tyrosine  
 tyrosine charcoal  
<sup>3</sup>H  
 (500/μl ) scintilla-  
 tion cocktail

5. Western blot analysis  
 trypsin hemacytometer  
 1 × 10<sup>6</sup> 100 μl SDS lysis  
 buffer(62.5 mM Tris, pH 6.8, 10% glycerol, 5%  
 -mercaptoethanol, 2.3% SDS) 10 soni-  
 cation . SDS-PAGE 10%, stacking gel pH  
 6.8, running gel pH 8.8 gel 5  
 20/μl(2 × 10<sup>5</sup> cell ) glycine  
 running buffer(200 mM glycine, 30/mM Tris-B,  
 pH 8.3, 1% SDS) 60/V 2-3  
 gel Towbin's (192/mM glycine,  
 25/mM Tris, pH 8.3, 0.1% SDS, 10% methanol)  
 400/mA 1  
 5% skim milk TBST (10/mM Tris,  
 pH 8.0, 150/mM NaCl, 0.1% Triton × 100  
 block 1:1000-1:4000 1  
 TBST  
 4 (5 , 5 , 15 , 15 ) HRP가  
 conjugate 2 (1:2000, Amer-sham)  
 4  
 ECL(Amersham) kit

6. 1 × 10<sup>6</sup> THGC 3-6  
 가 가  
 GTPCH I 2,4-diamino-6-hydroxypyrim-  
 idine(DAHP, 2.5/mM) sepiapterine reductase  
 N-acetylserotonin(NAS, 1 mM) 가

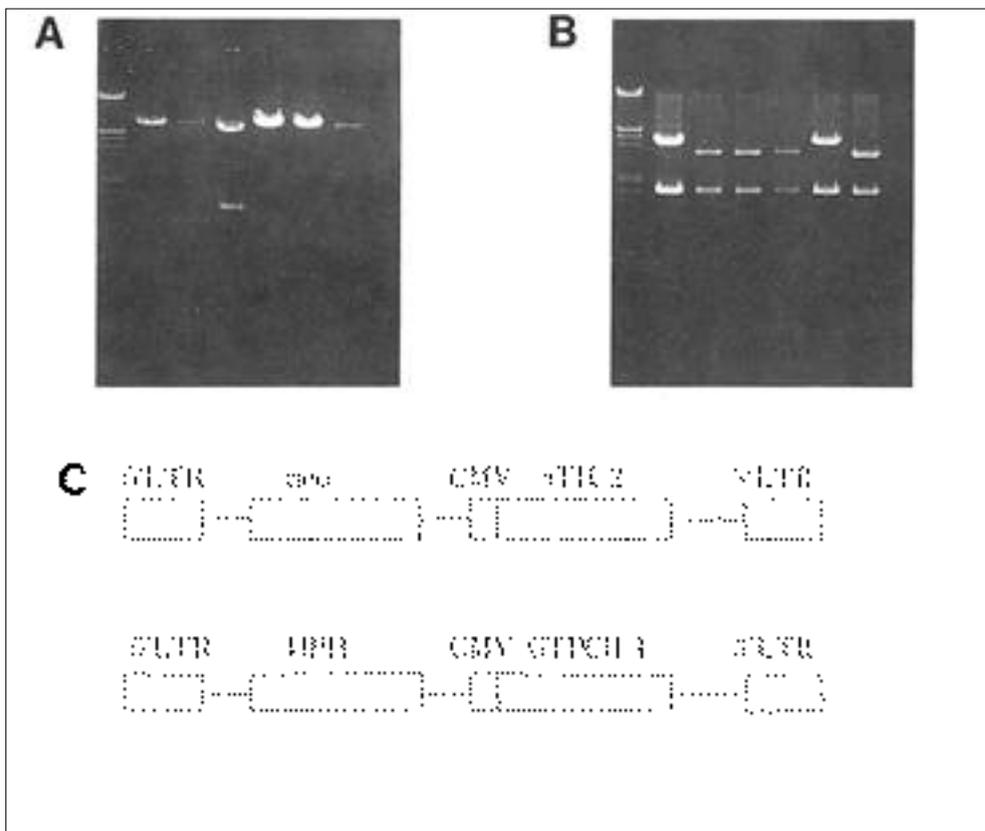
trypsin trypan blue

1. retroviral vector  
 hTH63 Bst XI/Klenow Hind  
 1.7 kb TH DNA Cla I/Klenow Hind  
 pLNCX . Ampicillin  
 colony alkaline lysis miniprep  
 plasmid DNA Bam HI Xho I  
 colony가  
 (Fig. 1A). GTPCH I Bam HI  
 Hinc 0.9 kb hygromycin-  
 B resistant marker 가 p gHC Cla  
 I/Klenow Bgl vector  
 colony alkaline lysis  
 miniprep Eco RI sub-  
 cloning (Fig. 1B).  
 figure 1C

2. TH clone  
 TH/pLNCX retroviral packaging cell line  
 BOSC 23 transfection retro-  
 virus . retrovirus NIH-  
 3T3

subcloning ring single colony  
 12 colony KNTH1 KNTH12  
 KNTH6 KNTH11  
 10  
 10<sup>6</sup> 100/μℓ lysis  
 가 20/μℓ 10% PAGE-SDS  
 anti-TH blot (Fig. 2).  
 NIH-3T3 TH  
 (lane 1) NIH-3T3  
 TH (lane 2-11).  
 KNTH1, KNTH4, KNTH8, KNTH12가 가 TH  
 KNTH2, KNTH5, KNTH4  
 KNTH7, KNTH9, KNTH10  
 TH

3. TH GTPCH clone  
 In vivo TH tetrahydro-  
 biopterin(BH<sub>4</sub>) TH  
 TH KNTH4  
 GC/p gHC virus  
 KNTH4 clone  
 colony 3  
 12 colony  
 KNTH4GC1 KNTH4GC12  
 12 colony 8 colony 가  
 4 colony



**Figure 1.** Subcloning of Tyrosine hydroxylase and GTP cyclohydrolase I into pLNCX (A) and p gHC (B), respectively. A: One of the six plasmid DNAs digested with Bam HI and Xho I shows 1.2 kb fragment (lane 3) indicating a successful cloning. B: Two of the six plasmid DNAs digested with Eco RI show an increase of 3.2 kb to 4.1 kb (lane 1, 5) indicating a successful subclonings. Far left lanes in each panel is size markers ( DNA digested with Hin dIII and Eco RI). C: Schematics of functional units of recombinant retroviral vector plasmids having tyrosine hydroxylase (top) and GTP cyclohydrolase I (bottom).

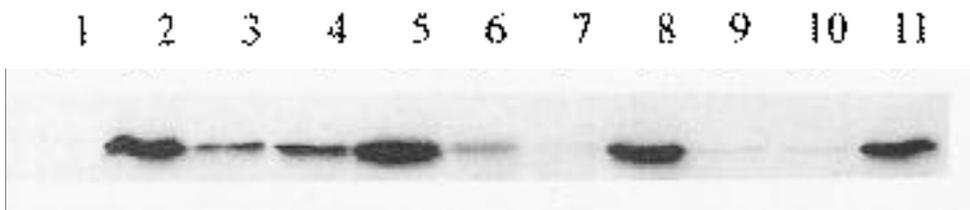
KNTH4GC3, KNTH4GC4, KNTH4GC9, KNTH4GC11  
가  
GC/p gHC GTPCH  
GTPCH I (Fig. 3A).  
KNTH4 infection  
가 GTPCH I

KNTH2GC anti-GTPCH  
GTPCH 가  
GTPCH (Fig. 3B).  
KNTH2GC4, KNTH2GC6, KNTH2GC7 가  
KNTH2GC1, KNTH2GC3,  
KNTH2GC5, KNTH2GC8 ,  
KNTH2GC10 가 GTPCH

TH KNTH2(Fig. 2,  
lane 3) GTPCH 12 colony  
KNTH2GC1-KNTH2GC12  
KNTH2HC2 가 KNTH4GC  
GTPCH (Fig. 3A, lane  
2) KNTH2GC9, KNTH2GC11, KNTH2GC12  
clone 가 8가

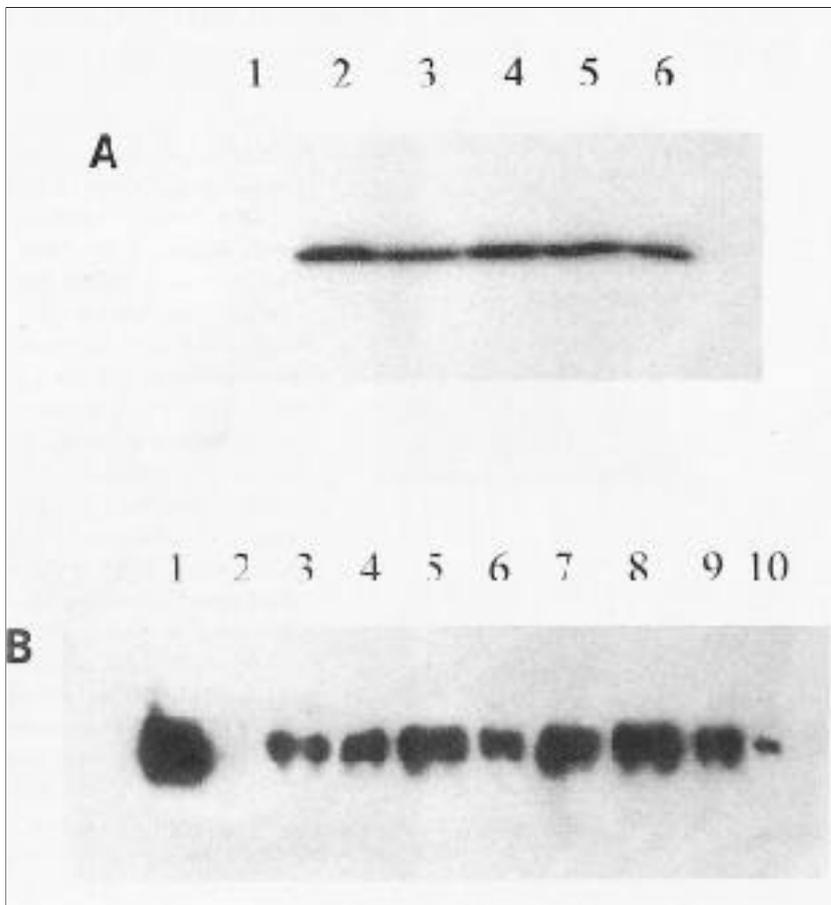
4. GTPCH I TH  
GTPCH I TH BH4가 TH  
KNTH2  
(Table  
TH가  
KNTH2 GTPCH I

가 tyrosine hydroxylation



**Figure 2.** Western blot analysis of 10 colonies (lane 2 through 11) of NIH-3T3 infected with recombinant TH/pLNCX virus. The immobilized samples were blotted with anti-TH antibody. The

first lane contains sample prepared from uninfected NIH-3T3 and following lanes contain samples from KNTH1, KNTH2, KNTH3, KNTH4, KNTH5, KNTH7, KNTH8, KNTH9, KNTH10, and KNTH12, respectively.



**Figure 3.** Western blot analysis of colonies from KNTH2 or KNTH4 infected with recombinant GC/p gHC virus. The immobilized samples were blotted with anti-GTPCH I antibody. A: Lane 1 shows negative control (KNTH2) and lane 2 through 6 show KNTH2GC2, KNTH4GC3, KNTH4GC4, KNTH4GC9, KNTH4GC11 cells, respectively. B: Lane 1 shows positive control, lane 2 shows negative control (KNTH2), and lane 3 through 10 are KNTH2GC1, KNTH2GC3, KNTH2GC4, KNTH2GC5, KNTH2GC6, KNTH2GC7, KNTH2GC8, KNTH2GC10, respectively.

KNTH2GC6 1/10-1/15 1/60 가 (98%) TH 3 (Fig. 4A, lane 2, 3, 4) KNTH2(Fig. 4A, lane 1) TH

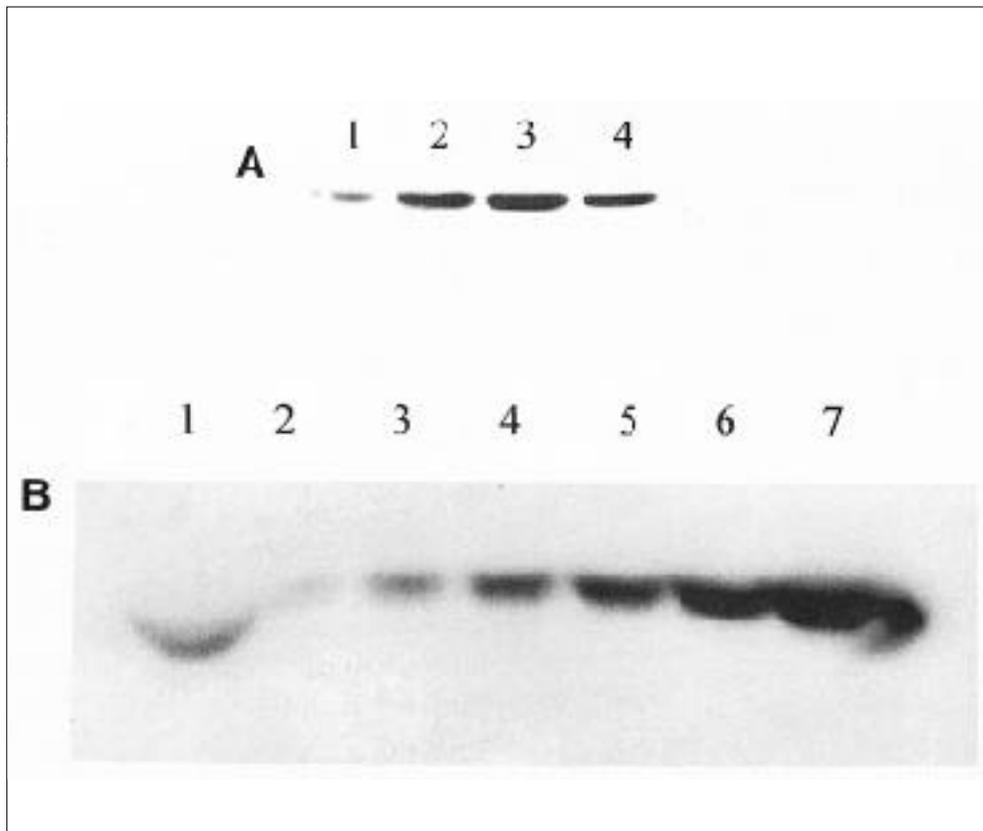
5. tetrahydrobiopterin TH TH biopterin 가 TH tetrahydro- KNTH2GC6 TH (Fig 4B). Fig. 4B lane 1 7 2 × 10<sup>6</sup> KNTH2 KNTH2GC6 Fig 4B lane 6 1 × 10<sup>6</sup>, Fig 4B lane 5 5 × 10<sup>5</sup> Fig. 4B lane 1 lane 3 lane 4 KNTH2GC6 KNTH2 10 TH

**Table 1.** TH activity assay of control and cells expressing TH alone or TH and GTPCH I

Sample	TH activity (nm/mg/min)
Striatum	0.0642
Hippocampus	0.0383
NIH-3T3	0.0000
KNTH2 (80% confluent)	0.0042
KNTH2 (98% confluent)	0.0070
KNTH2GC6 (80% confluent)	0.2688
KNTH2GC6 (80% confluent)	0.4036

TH: tyrosine hydroxylase  
GTPCH I: GTP cyclohydrolase I

6. TH BH<sub>4</sub>가 GTPCH I BH<sub>4</sub> phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase, nitric oxide synthase PC12 murine erythrocythemia mitogenic effect가



**Figure 4.** Comparison of expression level of tyrosine hydroxylase in cells expressing TH alone (lane 1) vs. cells expressing both TH and GTPCH I (lane 2 through 4). A: 10<sup>6</sup> cells from each cell lines were lysed in 100 μl of SDS lysis solution and 20 μl was loaded in each lane. SDS-PAGE separated samples were blotted with anti-TH antibody. Lane 1: KNTH2, lane 2: KNTH2GC5, lane 3: KNTH2GC6, lane 4: KNTH2GC8. B: Western blot analysis of 2.0 × 10<sup>6</sup> KNTH2 (lane 1), 6.25 × 10<sup>4</sup> KNTH2GC6 (lane 2), 1.25 × 10<sup>5</sup> KNTH2GC6 (lane 3), 2.5 × 10<sup>5</sup> KNTH2GC6 (lane 4), 5.0 × 10<sup>5</sup> KNTH2GC6 (lane 5), 1.0 × 10<sup>6</sup> KNTH2GC6 (lane 6), 2.0 × 10<sup>6</sup> KNTH2GC6 (lane 7). As in

Fig. A numbered cells were lysed in 100 μl of SDS lysis solution and 20 μl was loaded in each lane.

**Table 2.** Comparison of expression level of tyrosine hydroxylase in TH expressing cells vs. cells expressing both TH and GTPCH I.

Cell line	Densitometric reading
KNTH2	3576
KNTH2GC5	9877
KNTH2GC6	11253
KNTH2GC8	5000

TH: tyrosine hydroxylase  
GTPCH I: GTP cyclohydrolase I

**Table 3.** Growth rate of KNTH2GC6 at the presence or absence of inhibitors of BH4 biosynthesis.

Inhibitors	Cell number ( $\times 10^6$ )
No inhibitor	13.8
NAS	4.4
DAHP	6.8
NAS + DAHP	1.1

NAS: N-acetylserotonin  
DAHP: 2,4-diamino-6-hydroxypyrimidine

BH<sub>4</sub>가 1  $\times 10^6$  KNTH2GC6  
GTPCH I DAHP(2.5 mM) sepiapterin  
reductase NAS(1 mM)  
1 2 3  
trypsin trypan  
blue hemacytometer  
(Table 2). 가 KNTH2GC6  
3 1  $\times 10^6$  13.8  $\times 10^6$  24-  
30 NAS  
4.4  $\times 10^6$  DAHP 6.8  $\times$   
10<sup>6</sup> BH<sub>4</sub> 가  
NAS DAHP 가 3  
1.1  $\times 10^6$   
TH , K<sub>m</sub>, K<sub>i</sub>  
serine threonine 7가  
kinase 15  
kinase TH K<sub>m</sub> K<sub>i</sub> 15  
protease 가 39  
cofactor BH<sub>4</sub> TH  
TH specific phosphatase  
TH 40 protease  
가 TH  
TH 가

NIH-3T3 TH  
(Fig. 2 Fig.  
3) TH  
GTPCH I GTP  
BH<sub>4</sub> 41  
GTPCH I GTP가 7,8-dihydroneopterin  
triphosphate 2,4-diamino-6-  
hydroxypyrimidine(DAHP) inhibit 7,8-  
dihydroneopterin triphosphate 6-pyruvoyl  
tetrahydrobiopterin synthase 6-pyruvoyl  
tetrahydropterin sepiapterin  
reductase BH<sub>4</sub> 42 N-acetyl  
serotonin(NAS) 38  
NIH-3T3 GTPCH I BH<sub>4</sub>  
GTPCH I BH<sub>4</sub> 43  
TH  
가 KNTH2 KNTH2GC6  
trypsin hemacytomer  
western blot  
TH densitometric reading  
3가 KNTH2GC KNTH2  
1.4-3 (Fig 4A, Table 2) TH  
X-ray Fig 4B  
KNTH2  
KNTH2HC6 western blot  
exposure  
가 4  $\times$   
10<sup>5</sup> KNTH2 TH  
(Fig. 4B, lane 1) 5  $\times 10^4$  KNTH2GC6  
TH (Fig. 4B, lane 4) 2.5  $\times 10^4$   
KNTH2GC6 TH (Fig. 4B, lane 3)  
KNTH2GC6 KNTH2  
10 TH  
KNTH2GC6 KNTH2 60  
4-6  
10  
KNTH2GC6 6

KNTH2 KNTH2GC6  
 가 GTPCH 1 BH<sub>4</sub>  
 KNTH2GC6 가 BH<sub>4</sub>  
 BH<sub>4</sub>가 TH 가  
 가  
 Table 3  
 가 TH  
 BH<sub>4</sub>  
 cofactor가 가  
 가 GTP cyclohydro-  
 lase 1 DAHP sepiapterin reductase  
 NAS (Table  
 3). 1 × 10<sup>6</sup> KNTH2GC6 plate Table  
 2 3  
 BH<sub>4</sub>  
 TH BH<sub>4</sub>가  
 가 PD

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