

Supplementary materials

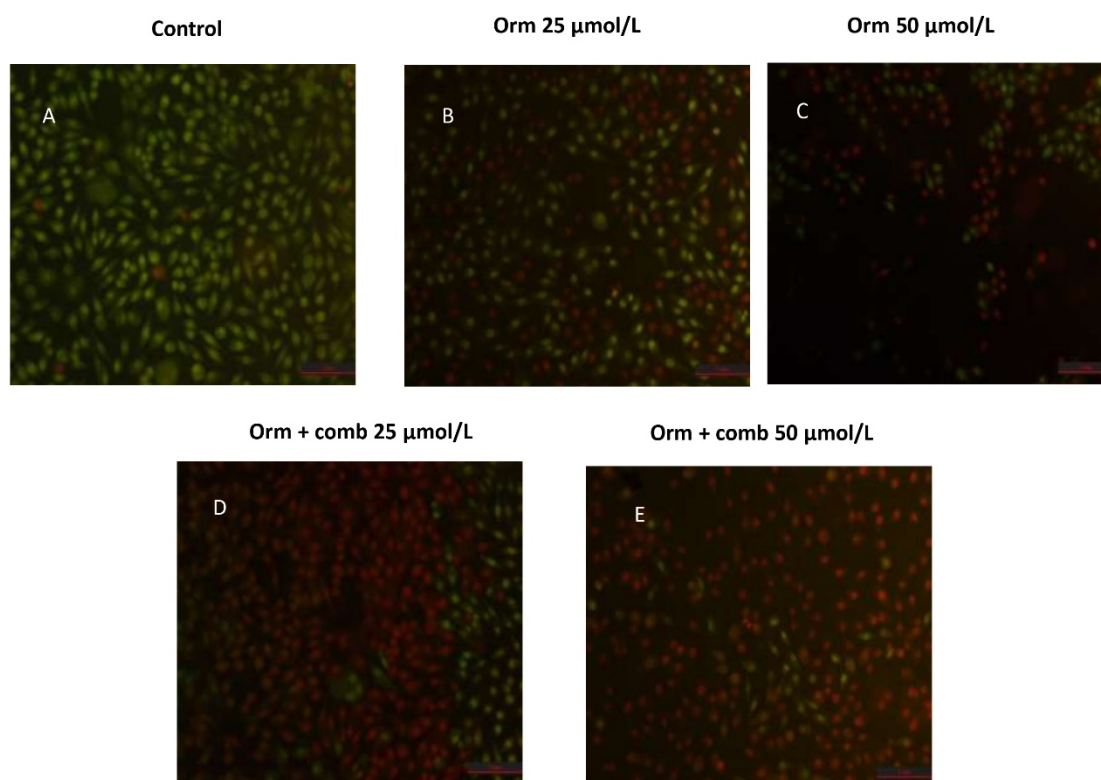


Figure S1. Nuclear morphological analysis by AO/EB staining. (A) Control appeared in green colour with uniform distribution; (B) Orm 25 $\mu\text{mol/L}$ which allowed EB to enter and stain the nuclei orange; (C) Orm 50 $\mu\text{mol/L}$: showed decreased cell number; (D) Orm + comb 25 $\mu\text{mol/L}$: the cells showed orange fluorescence by EB staining effectively; (E) Orm + comb 50 $\mu\text{mol/L}$ appeared orange indicates subsequent DNA damage

Primer information

The primers were designed using the online primer designing software, Primer3 (<https://primer3.ut.ee/>). Sequences downloaded in FASTA format files used along with subsequent parameters settings. Primer length: 20–26 bases; primer melting temperature around 60 to 64°C; and product size range: <250 bp for heat-shock proteins 70 (Hsp-70), Zinc finger E box binding Homeobox 1 (Zeb1), Vascular Endothelial Growth Factor (VEGF), Tumor Suppressor Protein (p53), Cyclin-dependent kinase inhibitor (p21), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Table S1: Sequences of primers used for the real-time PCR

Primer	Sequence	Accession number
<i>ZEB-1</i> (Forward)	TGCACTGAGTGTGGAAAAGC	NM_001128128.3
<i>ZEB-1</i> (Reverse)	TGGTGATGCTGAAAGAGACG	
<i>VEGF</i> (Forward)	ACGGTCCCTCTTGGAATTGG	M32977.1
<i>VEGF</i> (Reverse)	GGCCGCGGTGTGTCTA	
<i>P53</i> (Forward)	TGTGTCTGAGGGGTGAACG	U94788.1
<i>P53</i> (Reverse)	GGACGGTTGGTTCCTGAGTTA	
<i>P21</i> (Forward)	TCAGTTCCTTGTGGAGCCG	NM_001374511.1
<i>P21</i> (Reverse)	AGTCGAAGTTCCATCGCTCA	
<i>HSP70</i> (Forward)	TCCAGGAATCTGAAGAACGACC	L12723.2
<i>HSP70</i> (Reverse)	GGTGTGACTTCCATCCACTC	
<i>GAPDH</i> (Forward)	GGCAAATTCCATGGCACCGT	NM_001256799.3
<i>GAPDH</i> (Reverse)	GCATCGCCCCACTTGATTTT	

We used the following accession number sequence for the primer design and manually sequences of ZEB-1 (NM_001128128.3), VEGF (M32977.1), P53 (U94788.1), P21 (NM_001374511.1), HSP70 (L12723.2) & GAPDH (NM_001256799.3) were copy-pasted in Word document and checked each forward and reverse primer sequence alignment as shown in the word document attached in this e-mail. We used reverse complement sequences for reverse primer verification.

Primer	Sequence	Accession number
<i>ZEB-1</i> (Forward)	TGCACTGAGTGTGGAAAAGC	NM_001128128.3
<i>ZEB-1</i> (Reverse)	TGGTGATGCTGAAAGAGACG	
<i>ZEB-1</i> Reverse compliment for reverse primer:	CGTCTCTTTCAGCATCACCA	
<i>VEGF</i> (Forward)	ACGGTCCCTCTTGGAATTGG	M32977.1
<i>VEGF</i> (Reverse)	GGCCGCGGTGTGTCTA	
<i>VEGF</i> Reverse compliment for reverse primer:	TAGACACACCGCGGCC	

<i>P53</i> (Forward)	TGTGTCTGAGGGGTGAACG	U94788.1
<i>P53</i> (Reverse)	GGACGGTTGGTTCCTGAGTTA	
<i>P53</i> Reverse compliment for reverse primer:	TAACTCAGGAACCAACCGTCC	
<i>P21</i> (Forward)	TCAGTTCCTTGTGGAGCCG	NM_001374511.1
<i>P21</i> (Reverse)	AGTCGAAGTTCCATCGCTCA	
<i>P21</i> Reverse compliment for reverse primer:	TGAGCGATGGAAGTTCGACT	
<i>HSP70</i> (Forward)	TCCAGGAATCTGAAGAACGACC	L12723.2
<i>HSP70</i> (Reverse)	GGTGTGACTTCCATCCACTC	
<i>HSP70</i> Reverse compliment for reverse primer:	GAGTGGATGGAAGTCACACC	
<i>GAPDH</i> (Forward)	GGCAAATTCCATGGCACCGT	NM_001256799.3
<i>GAPDH</i> (Reverse)	GCATCGCCCCACTTGATTTT	
<i>GAPDH</i> Reverse compliment for reverse primer:	AAAATCAAGTGGGGCGATGC	

In addition, we have re-verified primer-blast software (link given in your attached Word file). We changed the setting as highlighted in below screenshot of database to **nr after that only sequences are given in the forward and reverse primer box and the results were found to be sequences compatible with VEGF (M32977.1), P53 (U94788.1) & HSP70 (L12723.2) as shown in subsequent screenshots.**

Settings change in the primer BLAST software

Exon junction span: No preference

Exon junction match: Min 5' match: 7, Min 3' match: 4, Max 3' match: 8

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min: 1000, Max: 10000

Note: Parameter values that differ from the default are highlighted in yellow

Primer Pair Specificity Checking Parameters

Specificity check: Enable search for primer pairs specific to the intended PCR template

Search mode: Automatic

Database: nr

Exclusion: Exclude predicted Refseq transcripts (accession with XM, XR prefix) Exclude uncultured/environmental sample sequences

Organism: Homo sapiens

Entrez query (optional):

Primer specificity stringency: Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end. Ignore targets that have 6 or more mismatches to the primer.

Max target amplicon size: 4000

Allow splice variants: Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

VEGF

Primer-BLAST > JOB ID:0tgMkA6KAYikGJMdn23L-Rmph3Jdb0Aya

Primer-BLAST Results

Input PCR template none
 Specificity of primers Target templates were found in selected database: Nucleotide collection (nt) (Organism limited to Homo sapiens)
 Other reports > Search Summary

Detailed primer reports

Primer pair 1


	Sequence (5'→3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACGGTCCCTCTTGGAAATTGG	20	59.67	55.00	4.00	2.00
Reverse primer	GCCCGGGGTGTGTCTA	16	58.93	68.75	6.00	2.00

Products on target templates
 >M32977.1 Human heparin-binding vascular endothelial growth factor (VEGF) mRNA, complete cds

product length = 111
 Forward primer 1 ACGGTCCCTCTTGGAAATTGG 20
 Template 868 887

Reverse primer 1 GCCCGGGGTGTGTCTA 16
 Template 978 963

TP53



Detailed primer reports

Primer pair 1

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGTGTCTGAGGGGTGAACG	Plus	19	19881	19899	59.25	57.89	3.00	2.00
Reverse primer	GGACGGTTGGTTCTCTGAGTTA	Minus	21	20106	20086	59.65	52.38	3.00	2.00

Product length 226

Products on intended targets
 >U94788.1 Human p53 (TP53) gene, complete cds

product length = 226
 Forward primer 1 TGTGTCTGAGGGGTGAACG 19
 Template 19881 19899

Reverse primer 1 GGACGGTTGGTTCTCTGAGTTA 21
 Template 20106 20086

Products on potentially unintended templates
 >CP139526.1 Homo sapiens isolate NA24385 chromosome 17

HSP70

Primer-BLAST > JOB ID:8PousAQzCZsupQygAcAoknVb0aBWYCK9Vw

Primer-BLAST Results

Input PCR template none
 Specificity of primers Target templates were found in selected database: Nucleotide collection (nt) (Organism limited to Homo sapiens)
 Other reports > Search Summary

Detailed primer reports

Primer pair 1

	Sequence (5'→3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCCAGGAATCTGAAGAAGCACC	22	59.77	50.00	5.00	0.00
Reverse primer	GGTGTGACTTCCATCCACTC	20	57.90	55.00	3.00	1.00

Products on target templates
 >L12723.2 Homo sapiens heat shock protein 70 (hsp70) mRNA, complete cds

product length = 191
 Forward primer 1 TCCAGGAATCTGAAGAAGCACC 22
 Template 2140 2161

Reverse primer 1 GGTGTGACTTCCATCCACTC 20
 Template 2330 2311

Human heparin-binding vascular endothelial growth factor (VEGF) mRNA, complete cds

GenBank: M32977.1

[GenBank Graphics](#)

>M32977.1 Human heparin-binding vascular endothelial growth factor (VEGF) mRNA, complete cds

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<i>VEGF</i> (Forward)	ACGGTCCCTCTTGGGAATTGG
<i>VEGF</i> (Reverse)	GGCCGCGGTGTGTCTA
Reverse compliment for reverse primer:	TAGACACACCGCGGCC

Human p53 (TP53) gene, complete cds

GenBank: U94788.1

[GenBank Graphics](#)

>U94788.1 Human p53 (TP53) gene, complete cds

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 TGG

<i>P53</i> (Forward)	TGTGTCTGAGGGGTGAACG
<i>P53</i> (Reverse)	GGACGGTTGGTTCCTGAGTTA
Reverse compliment for reverse primer:	TAACTCAGGAACCAACCGTCC

Homo sapiens heat shock protein 70 (hsp70) mRNA, complete cds

GenBank: L12723.2

[GenBank Graphics](#)

>L12723.2 Homo sapiens heat shock protein 70 (hsp70) mRNA, complete cds

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<i>HSP70</i> (Forward)	TCCAGGAATCTGAAGAACGACC
<i>HSP70</i> (Reverse)	GGTGTGACTTCCATCCACTC
<i>Reverse compliment for reverse primer:</i>	GAGTGGATGGAAGTCACACC