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| --- | --- | --- |
| **Gene (Accession No.)** | **Primer sequences (5’-3’)** | **Reasons for discard** |
| **Bcl2**  (NM\_016993.1) | F:GATTGTGGCCTTCTTTGAG | PMID16085054. Specificity not demonstrated by any of gradient annealing temperatures tested by PCR. Some changes has been done in reverse primer in order to become *in silico* specific. |
| R: CAAACTGAGCAGAGTCTTC |
| **Dkk1**  (NM\_001106350.1) | F:GGGAATTACTGCAAAAACGGAATA | Based on PMID[27216891](https://www.ncbi.nlm.nih.gov/pubmed/27216891). Only unspecific bands detected within annealing temperature range of 51-54.8C. |
| R: ATGATCGGAGGCAGACAGAG |
| F: ATGCCCTCTGACCACAGCCATT | PMID22133691. Only unspecific bands detected within annealing temperature range of 51-54.8C. |
| R: CACCGTGGTCATTGCCAAGGT |
| F: ATTCCAGCGCTGTTACTGTG | PMID27468227. Specificity not demonstrated by any of gradient annealing temperatures tested by PCR. |
| R: GAATTGCTGGTTTGATGGTG |
| **iNOs**  (NM\_012611.3) | F: GACCAGAAACTGTCTCACCTG | PMID22260992. Primers not specific. Other bands detected in agarose gel |
| R: CGAACATCGAACGTCTCACA |
| **p53**  (NM\_030989.3) | F: ATGGAGGATTCACAGTCGGATA | Based on PMID10889512. Primers not specific. Other bands detected in agarose gel |
| R: GACTTCTTGTAGATGGCCATGG |
| **Vegfa**  (ENSRNOG00000019598) | F: GTCCTGTGTGCCCCTAATGC | PMID15829992. Specificity not demonstrated by any of gradient annealing temperatures tested by PCR. |
| R: CGCTCTGAACAAGGCTCACAGT |

**S1 Table.** List of the primer pairs not validated successfully.