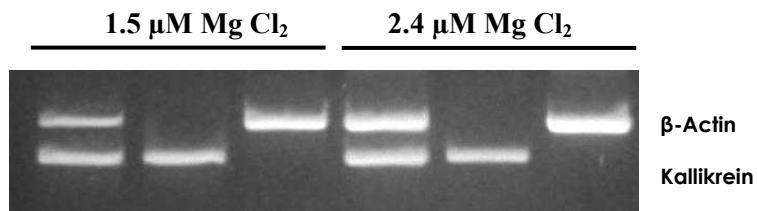


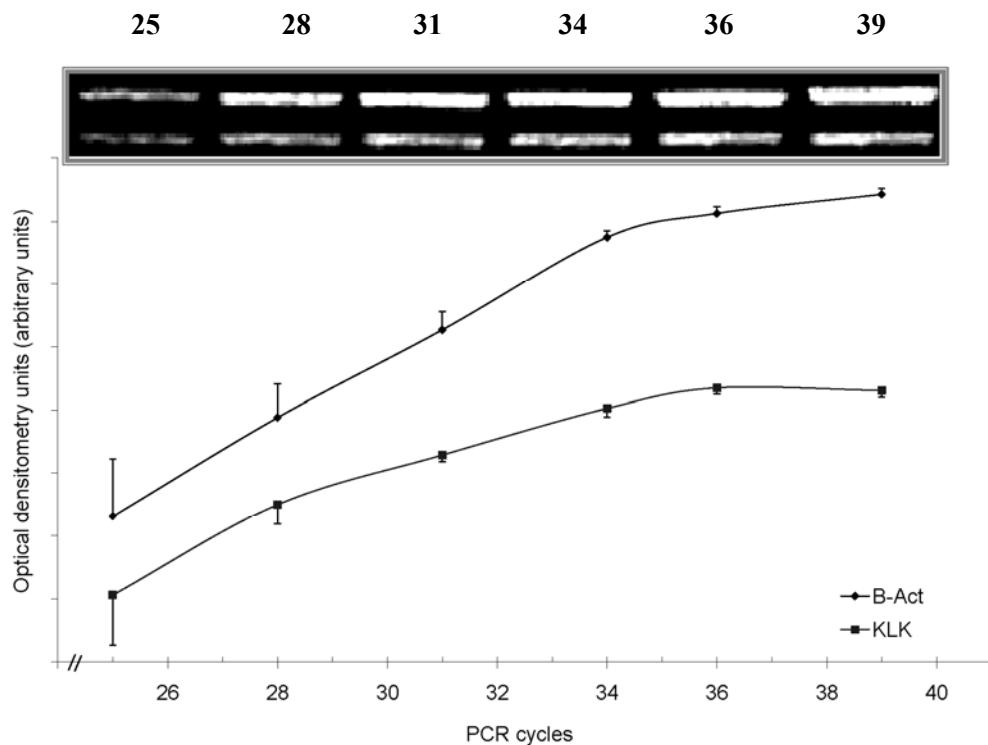
SUPPLEMENTARY DATA:

In order to improve and optimize the PCR procedure, several control experiments were made according to Marone *et al* [1] to ensure a semi quantification analysis:

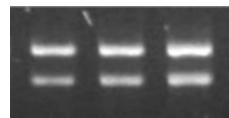
- Primers competency assay at different Magnesium levels:



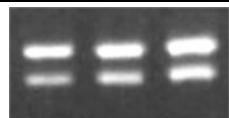
- Amplification curve for β -Actin (B-Act) and kallirein (KLK) accross different PCR cycles using male sham cDNA as template (example):



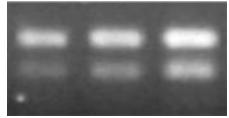
- Examples of semiquantification in different groups at 27, 29 and 31 PCR cycles:



oRx 1	β-Actin	KLK	KLK/ β-Actin
C 27	173.119	124.649	
C 29	185.318	145.091	0,783
C 31	201.687	167.056	



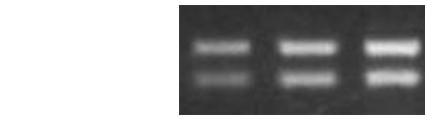
F 2	β-Actin	KLK	KLK/ β-Actin
C 27	196.643	142.410	
C 29	207.063	164.016	0,792
C 31	221.520	187.427	



M 3	β-Actin	KLK	KLK/ β-Actin
C 27	162.190	101.717	
C 29	182.896	119.446	0,728
C 31	207.826	154.016	



oVx 3	β-Actin	KLK	KLK/ β-Actin
C 27	112.639	102.069	
C 29	140.959	131.699	0,934
C 31	155.797	156.821	



oVx 4	β-Actin	KLK	KLK/ β-Actin
C 27	99.355	85.576	
C 29	114.688	110.616	0,964
C 31	138.484	129.045	

Abbreviations: M: male sham, F: female sham, oRx: orchidectomized and oVx: ovariectomized.

C 27, C29 and C 31: 27, 29 and 31 PCR cycles, respectively.

Reference:

1. Maria Marone, Simona Mozzetti, Daniela De Ritis, Luca Pierelli, Giovanni Scambia: Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biol. Proced. Online* 2001;3(1): 19-25.
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