

Article

Genetically Encoded Sensor Cells for the Screening of Glucocorticoid Receptor (GR) Effectors in Herbal Extracts

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Protein sequence

GR-Npuc-mNESc (1)

Expected Mass (kDa): 90.57

Sequence:

MDSKESLTPGREENPSSVLAQERGDVMDFYKTLRGGAT-
VKVSASSPSLAVASQSDSKQRRLLVDFPKGSVSNAQQPDLSKAVSLSMGLYMGETETK
VMGNDLGFPQQGQISLSSGETDLKLEESIANLNRSTSVPEN-
PKSSASTAVSAAPTEKEFPKTHSDVSSEQQHLKGQTGTNGGNVKLYTTDQSTFDILQD
LEFSSGSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNEDCK-
PLILPDTKPKIK-
DNGDLVLSSPSNVTLTPQVKTEKEDFIELCTPGVIKQEKLGTVYQCASFPGANIIGNKMS
AIVHGVSTSGGQMYHYDMNTASLSQQQDQKPIFNVIPPIVGSSENWN-
RCQGSDDNLTSLGTLNFPGRTVFSNGYSSPSMRPDVSSPPSSSTATTGPPPKLCLVCS
DEASGCHYGVLTCGSKVFFKRAVEGQHNYLCAGRNDCCIIRKKNCPACRYRK-
CLQAGMN-
LEARKTKKKIKGIQQATTGVSQETSENPNGNKTIVPATLPQLTPTLVSLLEVIEPEVLYAG
YDSSVPDSTWRIMTTLNMLGGRQVIAAVKWAKAIPG-
FRNLHLDDQMTLLQYSWMFLMAFAL-
GWRSYRQSSANLLCFAPDLIINEQRMTPCMYDQCKHMLYVSSELHRLQVSYEEYLC
MKTLLLLSSVPKDGLKSQELFDEIRMTYIKELGKAIVKREGNSSQNWQRFYQLT-
KLLDSM-
HEVVENLLNYCFQTFLDKTMSEFPEMLAEIITNQIPKYSNGNIKKLLFHQKLEIKIATR
KYLKGQNVYDIGVERDHNFAKNGFIASNCFNLSL

mCherry-mNES_N-Np_{UN}-NLS (2)

Expected Mass (kDa): 44.29

Sequence:

MVSKGEEDNMAIIEKFMRFKVHMEGSVNGHE-
FEIEGEGEGRPYEGTQAKLKVTGKG-
PLPFAWDILSPQFMYGSKAYVKHPADIPDYKLKSFPEGFKWERVMNFDGGVVTVTQ
DSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRL-
KLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQHERAEGRHS
TGGMDELYKPRKVYPILRLCLSYETEILTVEYGLLPYGKIVEKRIECTVYSVDNNGNI-
YTQPVAQWHDRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMR
VDNLNPNIKIATRKYLGKQNVYDIGVERKRPAATKKAGQAKKKKLD

mCherry-mNES_N-mNp_{UN}-NLS (3)

Expected Mass (kDa): 44.25

Sequence:

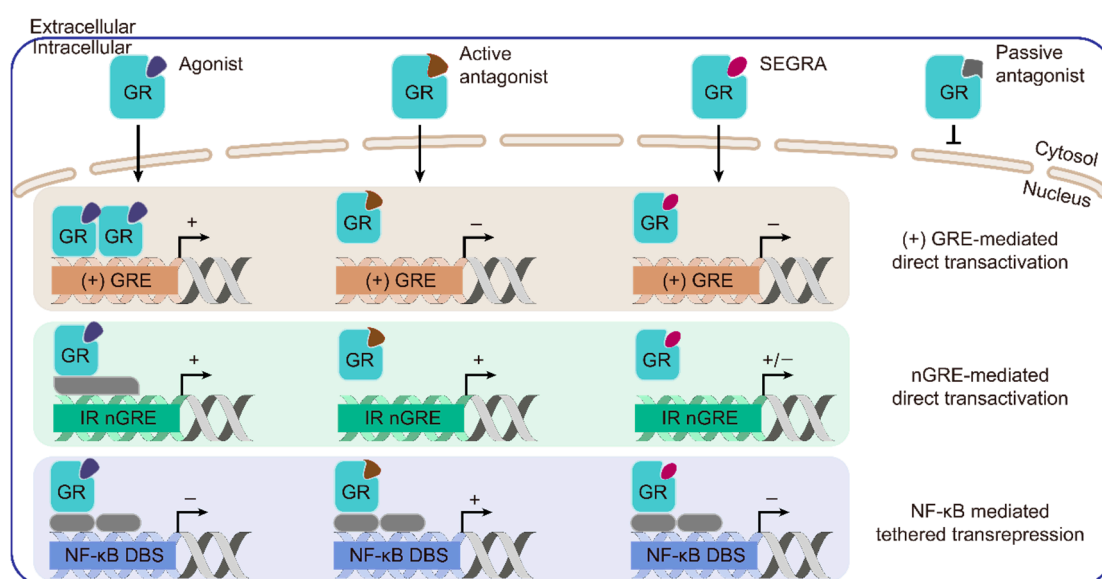
MVSKGEEDNMAIIEKFMRFKVHMEGSVNGHE-
FEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYG-
SKAYVKHPADIPDYLKLSFPEGFKWERVMNFDGGVVTVTQDSSLQDGEFI-
YKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRL-
KLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNI-
KLDITSHNEDYTIVEQHERAEGRHSTGGMDELYKPRKVYPILRLALSYTEILTVEY-
GLLPYGKIVEKRIECTVYSVDNNGNIYTQPVAAQWHDRGEQEVFEYCLEDGSLIRAT-
KDHKFMTVDGQMLPIDEIFERELDLMRVDNLPNIKIATRKYL-
GKQNVYDIGVERKRPAATKKAGQAKKKKLD

GR-Npuc-mNEsc-2xFLAG (4)

Expected Mass (kDa): 92.72

Sequence:

MDSKESLTPGREENPSSVLAQERGDVMDFYKTLRGGAT-
VKVSASSPSLAVASQSDSKQRRLLVDFPKGSVSNAQQPDLSKAVSLSMGLYMGE-
TETKVMGNDLGFPQQGQISLSSGETDLKLLSESIANLNRSTSVPEN-
PKSSASTAVSAAPTEKEFPKTHSDVSSEQQHLKGQTGTNGGNVKLYTTDQSTFD-
ILQDLEFSSGSPGKETNESPWRSDDLIDENCLLSPLAGEDDSFLLLEGNSNEDCK-
PLILPDTKPKIKDNG-
DLVLSSPSNVTLTPQVKTEKEDFIELCTPGVIKQEKLGTVYQCASFPGANIIGNK-
MSAISVHGVSTSGGQMYHYDMNTASLSQQQDQKPIFNVIPPIPVGSENWN-
RCQGSDDNLTSLGTLNFPGRTVFSNGY-
SSPSMRPDVSSPPSSSSTATTGPPPKLCLVCSEASGCHYGVLTCGCKVFFKRAVE-
GQHNYLCAGRNDCHDKIRRNCPACRYRKCLQAGMNLEARKTKKKIKGIQQATT-
GVSQETSENPKNKTIVPATLPQLTPTLVSLLEV-
IEPEVLYAGYDSSVPDSTWRIMTTLNMLGGRQVIAAVKWAKAIPG-
FRNLHLDDQMTLLQYSWMFLMAFALGWRYSYRQSSANLLCFAPDLI-
INEQRMTLPCMYDQCKHMLYVSSELHRLQVSY-
EEYLCMKTLTLLSSVPKDGKLSQELFDEIRMTYIKELGKAIV-
KREGNSSQNWQRFYQLTKLLDSMHEVVENLLNYCFQTFDKTMSIEFPEMLAEIIT-
NQIPKYSNGNIKKLLFHQKLEIKIATRKYLGKQNVYDIGVERDHNFALKNG-
FIASNCFNLSASDYKDDDDKDYKDDDDK



Scheme S1. A schematic illustration of the mode of GR gene regulation. Plus (+) indicates stimulation of gene expression and minus (-) in GRE or nGRE mediated GR transactivation suggests no effect on gene while the minus (-) in NF-κB mediated tethered transrepression implies repression of gene expression.

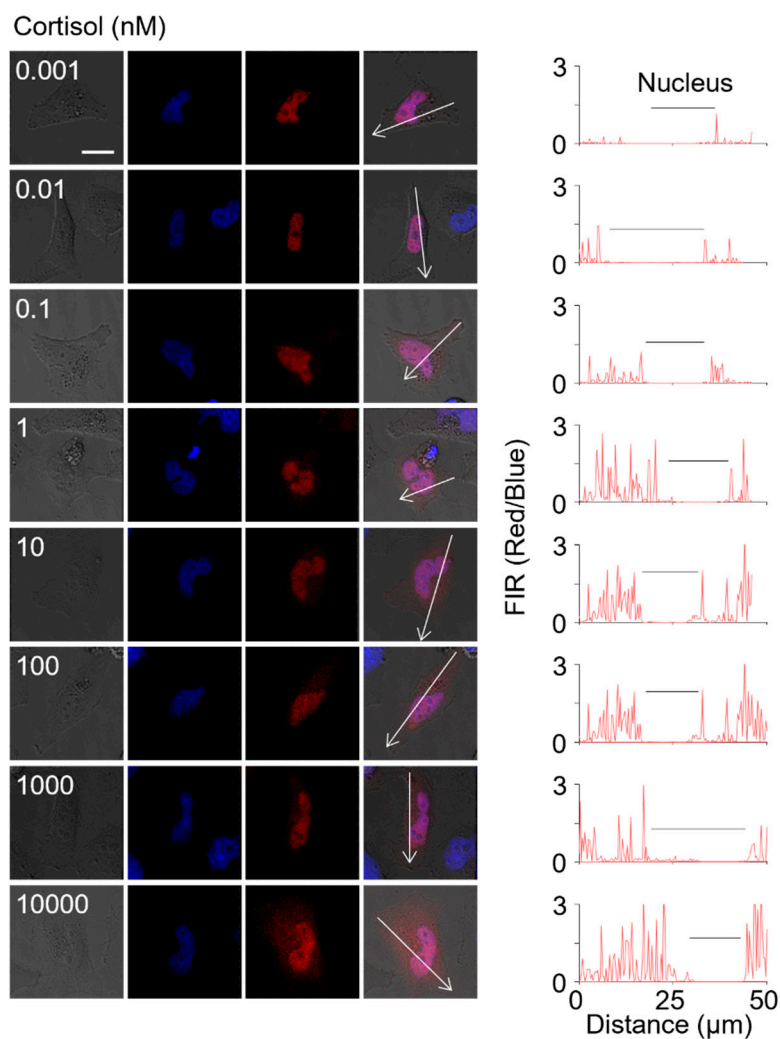


Figure S1. Dose-dependent treatment of cortisol to sensor cells. Cortisol was treated to the sensor cell by concentration and analyzed (scale bar = $20\mu\text{m}$).

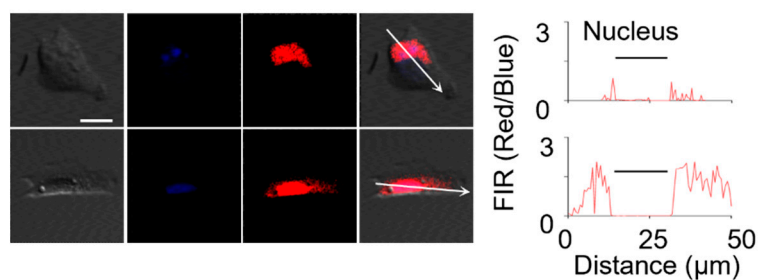


Figure S2. Detection of salivary cortisol using sensor cell. The sensor cells responded to Cort in human saliva with estimated Cort-concentration of 13 nM.

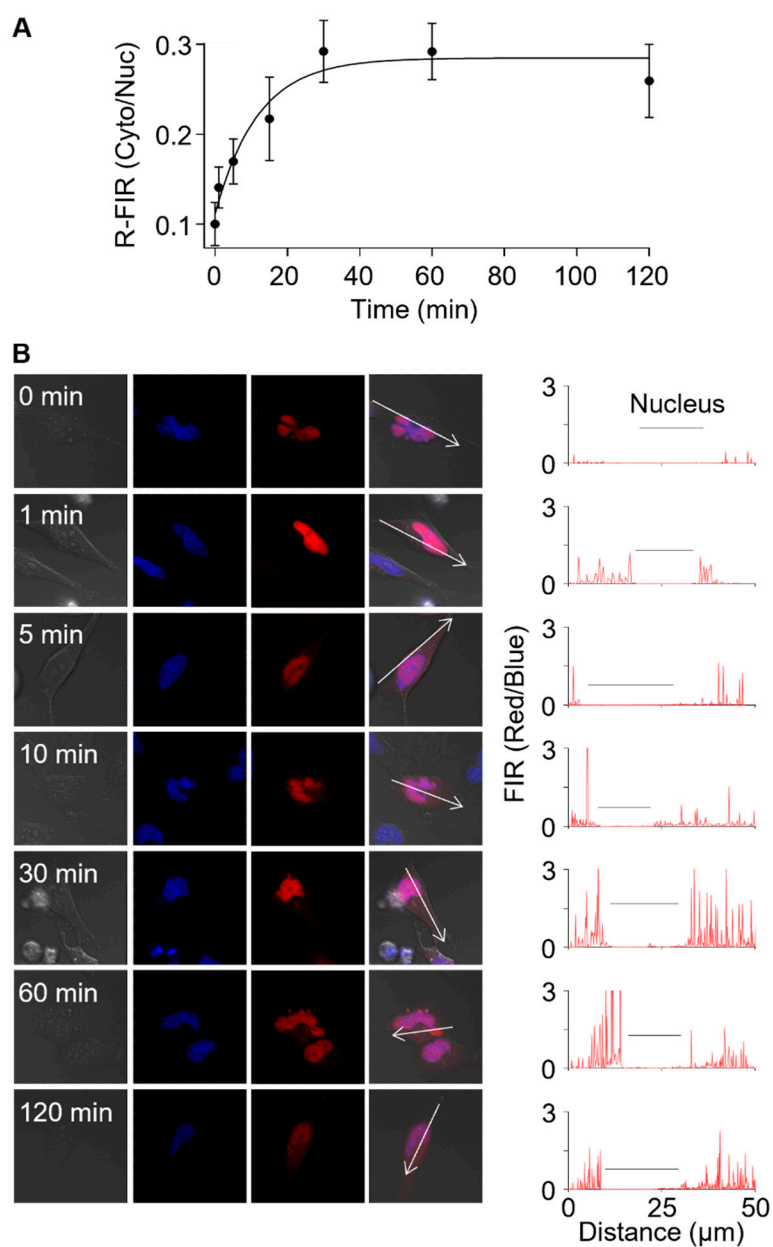


Figure S3. Time-dependent treatment of cortisol to sensor cells. (A) Time-dependent of R-FIR Cyto/Nuc signal was plotted and the limit of detection. (B) The sensor cells were treated with 10 μM of cortisol depending on time. (scale bar = 20 μm)..

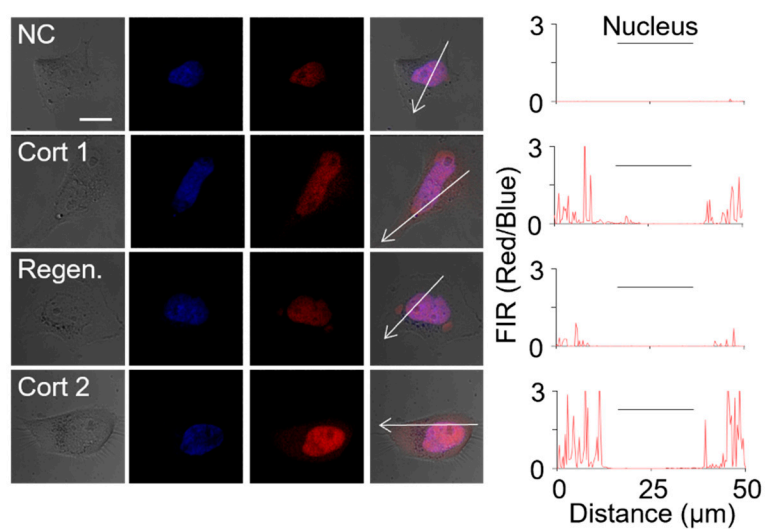


Figure S4. Renewability of sensor cell. Sensor cells were exposed to repetitive 10 μ M of cortisol with time intervals (scale bar = 20 μ m).

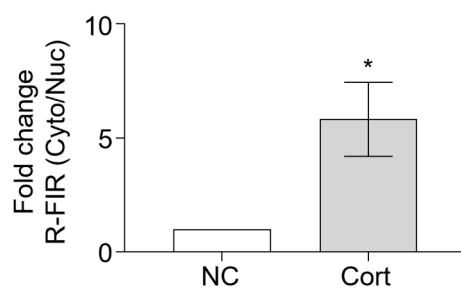


Figure S5. Cortisol sensing using HEK293T derived sensor cell. HEK293T-derived sensor cells successfully respond to cortisol and data were analyzed by unpaired T-test (* $p < 0.05$).

Table S1. Table of medicinal plants and extraction conditions.

Common name	Biological name	ratio (w/v) ^a	time (h)
Gogi berry	Lycium chinense	1:10	2.5
Cornelian berry	Cornus officinalis	1:10	3
Black raspberry	Rubus coreanus	1:2	3
Tumeric	Curcuma longa	1:10	2
Dong-quai	Angelica gigas	1:6	3
Ginseng ^b	Panax ginseng	-	-
Hasuo	Polygonum muliflorum	1:12.5	3
Licorice	Glycyrrhiza uralensis	1:10	3
Deer antler ^b	Cervus	-	-
Red ginseng	Panax ginseng	1:10	24
Reishi mushroom	Ganoderma lucidum	1:20	24
Red bean	Vigna angularis	1:20	24
Onion	Allium cepa	1:10	12
Garlic	Allium sativum	1:10	3

^a Ratio of the weight of the natural product and the volume of 70 % ethanol^b Purchased from Dongguk University Medical Center (Goyang, Korea)