Certificate of Analysis ER-β Competitor Assay, Red, 1 Kit



Part Number: P3032 Lot Number: 1099296 Immediate Storage: -80°C Shipping Conditions: dry ice 5791 Van Allen Way Carlsbad, CA 92008 Phone: 760.603.7200 Fax: 760.602.6500 www.lifetechnologies.com

Components:

| Description | Composition | Quantity | Part Number | Lot Number |
|--|--|-----------|-------------|------------|
| ER Red Assay Buffer | Buffer (pH 8.0) containing protein stabilizing agents and 10% glycerol. | 25 mL | P3031 | 36952GG |
| Fluormone™ EL Red | 200 nM in 20 mM Tris, 90% Methanol | 100 μL | P3030 | 35367FF |
| Estrogen Receptor-β, Human Recombinant | Estrogen Receptor-β, Human Recombinant, full length untagged in 50 mM BIS-TRIS-Propane (pH 9.0), 400 mM KCL, 2mM DTT, 1 mM EDTA and 10% Gycerol. | 750 pmols | P2466* | 919685B |

^{*}See individual COA

Storage and Handling: The performance of this product is guaranteed for 6 months from the date of purchase if stored and handled properly.

| Description | Storage and Handling | |
|--|--|--|
| ER Red Assay Buffer | 20-30°C. Mix by inversion prior to use. | |
| Fluormone™ EL Red | –20°C. Vortex prior to use. The Fluormone™ EL Red may have estrogenic activity in vivo and therefore should be handled with caution. | |
| Estrogen Receptor-β, Human Recombinant | Store at -80°C. At first use, aliquot and store at -80°C to avoid multiple freeze-thaws. Mix gently, do not vortex. If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase. | |

QUALITY ASSURANCE

Functional Testing:

Kit components are functionally tested for performance in the assay using a titration of a known agonist or antagonist.

Assay Specifications:

Using the conditions described in the kit protocol and keeping the plates in the dark and covered to prevent evaporation this kit has the following specification(s):

| Specification | Value | - 1 |
|----------------------------|----------|-----|
| AmP (0-100% inhibition) | > 100 mP | I |
| IC ₅₀ Estradiol | < 40 nM | j |

Manufacturing Specifications:

The concentration of Fluormone™ EL Red was determined by measuring its fluorescent intensity.

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Certificate of Analysis Estrogen Receptor-β, Human Recombinant, 750 pmols



Part Number: P2466 Lot Number: 919685B Immediate Storage: -80°C Shipping Conditions: dry ice 5791 Van Allen Way Carlsbad, CA 92008 Phone: 760.603.7200 Fax: 760.602.6500 www.lifetechnologies.com

Description:

Estrogen Receptor-β, Human Recombinant, full length untagged.

Activity Testing:

The concentration of active receptor is **4600 nM** as determined by quantitation of [6, 7–³H 9 (N)]–Estradiol receptor complexes using a hydroxylapatite (HAP) assay. Using the protein concentration as determined by Bradford, the specific activity is **13900 pmol/mg**.

Concentration:

 $0.33~{\rm mg/mL}$ total protein as measured using the Bradford protein assay with BSA as a standard.

Assay Concentration(s):

10 nM Estrogen Receptor-β, Human Recombinant is the final concentration recommended for the Polar Screen™ ER-β Competitor Assay, Green (P2700).

30 nM Estrogen Receptor–β, Human Recombinant is the final concentration recommended for the ER-β Competitor Assay, Red (P3032).

Aliases:

ESR2, NR3A2, ERB

Storage and Handling:

Store at -80°C. At first use, aliquot and store at -80°C to avoid multiple freeze-thaws. **Mix gently, do not vortex.** If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase.

Estrogen Receptor may aggregate with rough handling. Thaw in room temperature water bath and return to 4°C.

Storage Buffer:

50 mM BIS-TRIS-Propane (pH 9.0), 400 mM KCL, 2mM DTT, 1 mM EDTA and 10% Gycerol.

QUALITY ASSURANCE

Functional Testing:

The performance of each lot of Estrogen Receptor– β , Human Recombinant is confirmed in the following assay(s):

Polar ScreenTM ER- β Competitor Assay, Green (P2700). The ligand Estradiol, [6, 7-3H 9 (N)] was shown to displace FluormoneTM ES2 Green from Estrogen Receptor- β , Human Recombinant.

ER-β Competitor Assay, Red (P3032), Estradiol, [6, 7-3H 9 (N)] was shown to displace Fluormone $^{\text{TM}}$ EL Red from Estrogen Receptor–β, Human Recombinant.

Gel Information for Estrogen Receptor– β , Human Recombinant

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex® gels (Catalog #: EC6025BOX).

Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 1 μg Estrogen Receptor–β, Human Recombinant

Lane 3: 2 μg Estrogen Receptor–β, Human Recombinant

Lane 4: 5 μg Estrogen Receptor–β, Human Recombinant

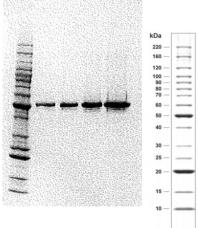
Lane 5: 10 μg Estrogen Receptor–β, Human Recombinant

Purity:

85% as determined by a Coomassie® blue stained SDS-PAGE gel.

Molecular Weight:

53.4 kDa. Calculated from the protein sequence(s).



Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_001428.1

1 MNYSIPSNYT NLEGGPGRQT TSPNYLWPTP GHLSPLVVHR QLSHLYAEPQ KSPWCEARSL EHTLPVNRET LKRKVSGNRC ASPVTGPGSK RDAHFCAVCS NP_001428.1

101 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 154 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRRKSCQA CRLRKCYEVG MVKCGSRRER CGYRLVRQR SADEQLHCAG KAKRSGGHAP 155 DYASGYHYGV WSCEGCKAFF KRSIQGHNDY ICPATNQCTI DKNRKSCH KAKLOWA KKIPGFVELS LFDQVRLLES CWMEVLMMG MWRSIDHPGK WWSIDHPGK W



Becky, Baker, QA Engineer II

Date: 01/May/2012

 $\label{eq:novexsigma} \mbox{Novex} (B) = \mbox{Solution} \mbox{Novex} (B) + \mbox{Solution} \mbox{Solution}$

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^{*} highlighted residues denote differences from the reference protein sequence(s).