

For use in quality control/
manufacturing process only.



Density Reference Standard Beads (DRSB)

 **Version 47**

Content version: March 2020

Beads for one-point density calibration

Cat. No. 06 422 659 001

Batch A

Store Beads at +2 to +8°C

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1. Introduction

The Cedex HiRes Analyzer measures the cell density of a cellular suspension, along with its viability status and cell characteristics such as diameter and compactness. In order to check the correct calibration with regard to the density, use the Density Reference Standard Beads instead of an ordinary cell sample.

This product is traceable in the following aspects:

- Particle Size: NIST, USA
- Particle Concentration: Physikalisch-Technische Bundesanstalt, Berlin, Germany

The Density Reference Standard Beads are made to mimic cell behavior in flow dynamics. Due to their size and optical properties, they will appear as dead cells in the Cedex Software.

Contents

Content	Volume	Cat. No.
Beads for one-point density calibration	10 ml	06 422 659 001

Storage and Stability

Store Beads at +2 to +8°C.

The product is stable until the expiry date printed on the label, when handled as described in these Instructions for Use.

2. How to Use this Product

There is no general advice with regard to how often or how many counts should be done in order to ensure that your analyzer is working correctly. Roche Diagnostics has had good results using the DRSB on a monthly basis, carrying out 10 samples per run with the Cedex HiRes Analyzer.

Two factors influence the acceptance range for calibration with Density Reference Standard Beads in connection with the Cedex HiRes Analyzer.

- **Sample preparation:** Mixing, pipette operation, and pipette quality (precision, accuracy, service state) have been shown in the field to add approximately 1.5 – 2% to the variability in density measurements.
- **Measurement precision is based on the statistical nature of the measurement process.** It depends on the density of the DRSB used, Cell Type parameter settings, and the level of precision used for the measurement.

Sampling quality is essential for the evaluation of the status of the instrument. Special care should be taken to ascertain, for example, that among other factors:

- Beads were not frozen, but stored properly at +2 to +8°C.
- Beads were allowed to acclimate to a temperature of +23 to +27°C prior to use.
- Weight of the unopened bottle is correct (see label on the bottle).
- Ultrasonic bath is used for mixing.
- Bottle is rocked gently, including rocking upside down.
- No more than 2 samples are drawn from the bottle without intermediate remixing.
- Only calibrated pipettes are used.
- Only trained staff are performing the sample preparation.

3. Protocol

3.1 Preparation of the DRSB solution

- Verify that the beads have been stored correctly at temperatures of +2 to +8°C (BEADS CANNOT BE FROZEN).
 - Verify that the bottle was securely closed before use (check the weight of the unopened bottle; the correct value is given on the bottle).
 - Allow the beads to acclimate to a temperature of +23 to +27°C prior to use.
 - Use an ultrasonic bath at a temperature of +23 to +27°C and at the highest available intensity for 5 minutes (with cap slightly loosened but secured against falling over) to shake the beads.
 - Ensure that no beads are sticking to the base or side of the bottle before use.
- Ⓢ The DRSB solution contains SDS, which may show signs of some coagulation or crystallization at low temperatures. Crystals and/or signs of coagulation can be removed by allowing the beads to acclimate, with occasional mixing, at +25°C until the coagulation disappears. Alternatively, the DRSB bottle can be gently rolled between the palms of the hands until the coagulation has disappeared. Note that as long as the DRSB solution has been allowed to acclimate to +23 to +27°C, and all steps in this Instructions for Use have been carried out, any remaining coagulation or crystallization will have no effect on the performance or quality of the DRSB solution when used in a Cedex HiRes Analyzer.

3.2 Checking the FlowFactor (FF) (see Figure 2)

- ① Pipet 1 sample of 0.3 ml DRSB into a Cedex Sample cup, and run the sample with factory settings for default Cell Type Std. Size immediately. Select the maximum possible setting for “precision”.
 - ② Mix the DRSB thoroughly, then pipet the next sample of 0.3 ml into a Cedex Sample cup, and run the sample immediately.
 - ③ Repeat this procedure until 10 samples are processed.
 - ④ Calculate the mean value of the Total Cell Density (TCD) of the 10 samples used.
 - ⑤ Calculate the relative standard deviation of the TCD values of the 10 samples used, and verify that the relative standard deviation is less than or equal to 5%. Otherwise, the Cedex HiRes Analyzer, the beads, or the handling have to be checked and the calibration must be repeated.
 - ⑥ Calculate the deviation of the mean TCD value of the 10 samples used from the actual value (given as Particle number/ml on the bottle of beads).
 - ⑦ Verify that the deviation of the mean TCD value is less than or equal to 5%, or as specified by your requirements, from the actual value given on the bottle for the beads. If the value falls outside of the acceptable range, skip to Step 9.
 - ⑧ Close bottle tightly and store beads at +2 to +8°C (BEADS CANNOT BE FROZEN). The current FF is correct and no change is necessary.
 - ⑨ If the mean value falls out of range, repeat Steps 1 to 5 using a second/different LOT (batch) of Density Reference Standard Beads. Continue with Step 10.
 - ⑩ Calculate the FlowFactor (FF) of each measurement series (see 5.1, “How to Calculate and Change the FlowFactor”), and the mean value of the two FFs.
 - ⑪ Verify that the deviation of the two FFs from the mean value of the FFs, are less than or equal to 5%. Otherwise, the Cedex HiRes Analyzer, the beads, or the handling have to be checked and the calibration must be repeated.
 - ⑫ Calculate the new FF (mean value of the FFs); (see 5.1, “How to Calculate and Change the FlowFactor”), or follow your company’s requirements.
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4. Lot Specific Data

Cat. No. 06 422 659 001, Batch A

Valid for Lot. No. 57130047

In this chapter, you will find lot specific data about your product. The table below provides the following information for each bottle produced for this lot.

Column 1: Bottle No. for the bottle.

Column 2: Actual concentration expressed in particle number/ml for the bottle.

Column 3: Total weight of bottle, including bottle, contents, and label.

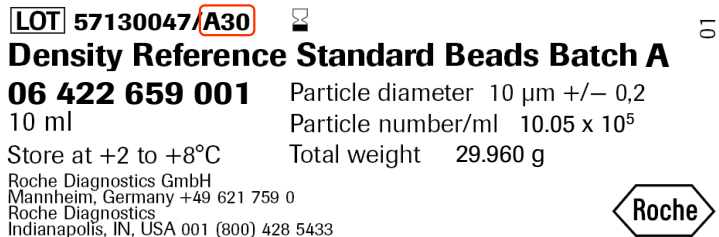
Column 4: Check Box for marking which bottle was received.


🕒 Use this table as follows:

- 1 Print out the table.
- 2 Find the bottle number on the bottle label as shown in Figure 1.
- 3 Place a check mark in the "Bottle Received" column to mark the specific bottle received for future reference.

🕒 This product is traceable in the following aspects:

- Particle Size: NIST, USA
- Particle Concentration: Physikalisch-Technische Bundesanstalt, Berlin, Germany



LOT 57130047/A30 

Density Reference Standard Beads Batch A 6

06 422 659 001 Particle diameter 10 µm +/- 0,2
10 ml Particle number/ml 10.05 x 10⁵

Store at +2 to +8°C Total weight 29.960 g

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Fig. 1: Example of how to find the bottle number on the bottle label. The bottle number is circled.

Lot Specific Data

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received	ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A1	10.19	29.526		A30	10.07	29.568	
A2	10.09	29.384		A31	10.01	29.547	
A3	10.14	29.398		A32	10.03	29.416	
A4	10.15	29.557		A33	10.09	29.414	
A5	10.11	29.511		A34	10.11	29.379	
A6	10.03	29.434		A35	10.12	29.625	
A7	10.03	29.509		A36	10.07	29.702	
A8	10.06	29.519		A37	9.93	29.512	
A9	10.08	29.454		A38	9.99	29.585	
A10	10.05	29.494		A39	10.08	29.470	
A11	10.06	29.449		A40	10.10	29.845	
A12	10.20	29.465		A41	10.04	29.411	
A13	10.14	29.505		A42	10.21	29.523	
A14	10.06	29.483		A43	10.19	29.463	
A15	10.08	29.591		A44	10.11	29.580	
A16	10.08	29.522		A45	10.08	29.605	
A17	10.10	29.444		A46	10.17	29.488	
A18	9.98	29.590		A47	10.14	29.503	
A19	10.15	29.625		A48	10.07	29.578	
A20	10.05	29.547		A49	10.22	29.452	
A21	10.06	29.538		A50	10.08	29.688	
A22	10.10	29.463		A51	10.18	29.340	
A23	10.08	29.482		A52	10.09	29.301	
A24	10.11	29.362		A53	10.14	29.410	
A25	10.07	29.512		A54	10.14	29.605	
A26	10.01	29.470		A55	10.12	29.626	
A27	10.11	29.570		A56	10.02	29.567	
A28	10.01	29.539		A57	10.05	29.633	
A29	10.07	29.479		A58	10.00	29.547	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received	ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A59	10.10	29.539		A88	10.05	29.521	
A60	10.09	29.517		A89	10.03	29.572	
A61	10.11	29.351		A90	10.06	29.641	
A62	10.15	29.430		A91	10.06	29.507	
A63	10.09	29.522		A92	10.07	29.451	
A64	10.19	29.541		A93	10.12	29.508	
A65	10.08	29.676		A94	10.06	29.457	
A66	10.07	29.580		A95	10.13	29.526	
A67	10.03	29.563		A96	10.04	29.577	
A68	10.13	29.569		A97	10.03	29.512	
A69	10.08	29.547		A98	10.07	29.538	
A70	10.07	29.648		A99	10.11	29.460	
A71	10.10	29.153		A100	10.01	29.591	
A72	10.18	29.623		A101	10.05	29.626	
A73	10.14	29.444		A102	10.10	29.624	
A74	10.10	29.525		A103	10.19	29.612	
A75	10.06	29.521		A104	10.06	29.505	
A76	10.18	29.436		A105	10.02	29.656	
A77	9.95	29.559		A106	10.17	29.591	
A78	10.05	29.573		A107	10.06	29.662	
A79	10.04	29.553		A108	9.99	29.565	
A80	9.98	29.492		A109	10.08	29.710	
A81	10.18	29.546		A110	9.97	29.590	
A82	10.06	29.660		A111	10.16	29.600	
A83	10.20	29.558		A112	10.20	29.652	
A84	10.06	29.497		A113	10.11	29.758	
A85	10.19	29.550		A114	10.13	29.622	
A86	10.11	29.402		A115	10.13	29.736	
A87	10.14	29.561		A116	10.04	29.713	

Lot Specific Data

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A117	10.01	29.580	
A118	9.98	29.570	
A119	10.19	29.767	
A120	10.13	29.801	
A121	10.11	29.525	
A122	10.14	29.692	
A123	10.10	29.606	
A124	10.05	29.520	
A125	10.17	29.726	
A126	10.15	29.662	
A127	10.13	29.580	
A128	10.03	29.655	
A129	10.18	29.498	
A130	10.05	29.658	
A131	10.13	29.666	
A132	10.08	29.687	
A133	10.14	29.566	
A134	10.05	29.458	
A135	10.06	29.620	
A136	10.13	29.572	
A137	10.21	29.606	
A138	10.12	29.637	
A139	10.16	29.580	
A140	10.01	29.616	
A141	10.16	29.694	
A142	10.05	29.499	
A143	10.09	29.572	
A144	9.99	29.463	
A145	10.07	29.735	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A146	9.96	29.473	
A147	9.98	29.441	
A148	10.08	29.551	
A149	10.10	29.539	
A150	10.17	29.639	
A151	10.09	29.421	
A152	10.18	29.282	
A153	10.22	29.332	
A154	10.03	29.637	
A155	10.10	29.588	
A156	10.10	29.622	
A157	10.13	29.551	
A158	10.06	29.594	
A159	10.12	29.492	
A160	10.02	29.603	
A161	10.05	29.328	
A162	10.13	29.459	
A163	10.10	29.458	
A164	10.11	29.461	
A165	10.15	29.616	
A166	10.12	29.505	
A167	10.11	29.570	
A168	10.03	29.518	
A169	10.07	29.708	
A170	10.07	29.490	
A171	10.11	29.570	
A172	10.04	29.602	
A173	10.11	29.508	
A174	10.06	29.575	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received	ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A175	10.10	29.626		A204	10.18	29.067	
A176	10.06	29.478		A205	10.16	29.145	
A177	10.05	29.596		A206	10.07	29.242	
A178	10.23	29.464		A207	10.13	29.301	
A179	10.06	29.507		A208	10.08	29.173	
A180	10.00	29.484		A209	10.00	29.250	
A181	10.15	29.559		A210	9.99	29.197	
A182	10.17	29.611		A211	10.11	28.905	
A183	10.19	29.563		A212	10.10	28.832	
A184	10.06	29.441		A213	10.20	28.721	
A185	10.17	29.521		A214	10.05	29.207	
A186	10.09	29.534		A215	10.09	29.229	
A187	10.06	29.428		A216	10.08	29.248	
A188	10.09	29.489		A217	10.09	29.213	
A189	10.10	29.532		A218	10.02	29.198	
A190	10.04	29.592		A219	10.00	29.180	
A191	10.07	29.466		A220	10.10	29.317	
A192	10.05	29.339		A221	10.17	29.184	
A193	10.06	29.347		A222	10.10	29.152	
A194	10.09	29.181		A223	10.18	29.290	
A195	10.10	29.226		A224	10.08	29.217	
A196	10.19	29.323		A225	10.08	29.230	
A197	10.10	29.483		A226	10.17	29.260	
A198	10.09	29.542		A227	10.04	29.206	
A199	10.16	29.622		A228	10.14	29.213	
A200	10.19	29.423		A229	10.01	29.218	
A201	10.07	28.871		A230	10.11	29.135	
A202	10.20	28.856		A231	10.09	29.124	
A203	10.16	28.709		A232	10.16	29.212	

Lot Specific Data

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A233	9.95	29.356	
A234	10.00	29.227	
A235	9.98	29.160	
A236	10.09	29.212	
A237	10.16	29.172	
A238	10.01	29.234	
A239	10.17	29.160	
A240	10.19	29.345	
A241	10.10	29.101	
A242	9.97	29.118	
A243	10.09	29.351	
A244	10.08	29.197	
A245	10.22	29.204	
A246	10.18	29.233	
A247	10.09	29.222	
A248	10.24	29.263	
A249	10.11	29.217	
A250	10.11	29.335	
A251	10.03	29.008	
A252	10.07	29.000	
A253	10.09	29.097	
A254	10.16	29.020	
A255	10.00	29.256	
A256	10.02	29.110	
A257	9.98	29.260	
A258	10.15	29.269	
A259	9.95	29.337	
A260	10.05	29.220	
A261	10.04	28.939	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A262	10.04	29.058	
A263	10.01	29.115	
A264	10.06	29.222	
A265	10.01	29.202	
A266	10.13	29.180	
A267	10.03	29.411	
A268	10.07	29.329	
A269	10.03	29.294	
A270	10.10	29.340	
A271	10.21	29.114	
A272	9.98	29.264	
A273	10.10	29.325	
A274	10.09	29.206	
A275	10.15	29.266	
A276	10.06	29.225	
A277	10.05	29.156	
A278	10.01	29.323	
A279	9.99	29.247	
A280	10.08	29.243	
A281	10.18	29.300	
A282	10.23	29.253	
A283	10.09	29.330	
A284	10.02	29.241	
A285	10.11	29.355	
A286	10.14	29.331	
A287	10.12	29.303	
A288	10.02	29.314	
A289	10.15	29.377	
A290	10.03	29.338	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received	ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A291	10.10	29.191		A320	10.12	29.512	
A292	10.09	29.391		A321	10.11	29.101	
A293	10.05	29.180		A322	10.22	29.255	
A294	9.98	29.222		A323	10.06	29.301	
A295	10.04	29.320		A324	9.94	29.234	
A296	10.04	29.315		A325	10.18	29.256	
A297	10.05	29.301		A326	10.06	29.090	
A298	10.09	29.307		A327	10.08	29.242	
A299	10.11	29.336		A328	10.09	29.040	
A300	10.19	29.478		A329	10.11	29.282	
A301	10.03	29.101		A330	10.07	29.229	
A302	10.17	29.103		A331	10.08	29.174	
A303	10.16	29.311		A332	10.07	29.272	
A304	10.14	29.155		A333	10.18	29.290	
A305	10.03	29.281		A334	10.02	29.198	
A306	10.13	29.076		A335	10.09	29.319	
A307	10.20	29.233		A336	10.04	29.159	
A308	10.13	29.223		A337	10.13	29.260	
A309	10.03	29.095		A338	10.26	29.261	
A310	10.03	29.164		A339	9.97	29.153	
A311	10.12	29.204		A340	10.11	29.174	
A312	10.14	28.987		A341	10.11	29.085	
A313	10.13	29.157		A342	10.05	29.223	
A314	10.12	29.093		A343	10.14	29.081	
A315	10.15	29.117		A344	10.09	29.218	
A316	10.06	29.035		A345	10.05	29.126	
A317	10.20	29.120		A346	10.07	29.132	
A318	10.11	29.339		A347	10.05	29.248	
A319	10.02	29.294		A348	10.18	29.182	

Lot Specific Data

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A349	10.22	29.166	
A350	10.07	29.209	
A351	10.10	29.314	
A352	10.18	29.195	
A353	10.04	29.175	
A354	10.04	29.176	
A355	10.04	29.355	
A356	10.08	29.402	
A357	10.05	29.221	
A358	10.09	29.265	
A359	9.96	29.300	
A360	9.95	29.303	
A361	10.19	29.088	
A362	10.17	29.280	
A363	10.16	28.976	
A364	10.23	29.151	
A365	9.98	29.296	
A366	10.02	29.460	
A367	10.18	29.305	
A368	10.07	29.390	
A369	9.91	29.224	
A370	10.03	29.199	
A371	10.17	29.169	
A372	10.16	29.394	
A373	10.15	29.769	
A374	10.19	29.269	
A375	10.14	29.197	
A376	10.17	29.468	
A377	10.12	29.323	

ID-Nr. LOT 57130047	Concentration Particle number/ml ($\times 10^5$)	weight (g)	Bottle received
A378	10.16	29.087	
A379	10.10	29.186	
A380	9.95	29.165	
A381	10.09	29.175	
A382	10.03	29.249	
A383	10.09	29.100	
A384	10.14	29.127	
A385	10.13	29.169	
A386	10.05	29.254	
A387	10.04	29.162	
A388	10.01	29.215	
A389	10.04	29.275	
A390	10.14	29.313	
A391	10.17	29.258	
A392	10.12	28.983	
A393	10.24	29.501	
A394	10.14	29.257	
A395	10.16	29.278	
A396	10.11	29.312	
A397	10.05	29.240	
A398	10.21	28.804	
A399	10.20	29.140	
A400	10.16	29.240	
A401	10.17	29.490	
A402	10.09	29.540	
A403	10.03	29.325	

5. Adjusting the FlowFactor

The FlowFactor (FF) is analyzer specific and part of the conversion factor that relates the number of objects detected in the Cedex HiRes Analyzer to the actual density in the analyzed sample.

The conversion factor is linearly dependent on the FlowFactor, thus allowing for the possibility to compute the appropriate setting for this parameter via the comparison of Cedex HiRes Analyzer results versus a known density of a sample (*e.g.*, Density Reference Standard Beads).

Refer to the relevant Cedex HiRes Operator's Manual for the location of the current FlowFactor. The location depends on the installed Software version.

5.1 How to Calculate and Change the FlowFactor

- 1 Write down the current FlowFactor (FFold) and calculate a new FlowFactor as follows:

$$FF(\text{new } 1) = \frac{\text{actual density (according to bottle label)}}{\text{mean value TCD of measurement series 1}} \times FF(\text{old})$$

$$FF(\text{new } 2) = \frac{\text{actual density (according to bottle label)}}{\text{mean value TCD of measurement series 2}} \times FF(\text{old})$$

("actual density" is specified as Particle number/ml on the label of the bottle of beads used for the calibration.)

$$FF(\text{new}) = \frac{FF(\text{new } 1) + FF(\text{new } 2)}{2}$$

- 2 Refer to the relevant Cedex HiRes Operator's Manual for information about the location of the FlowFactor. Update the FlowFactor in that location based on the result calculated in Step 1.

5.2 FlowFactor Calibration

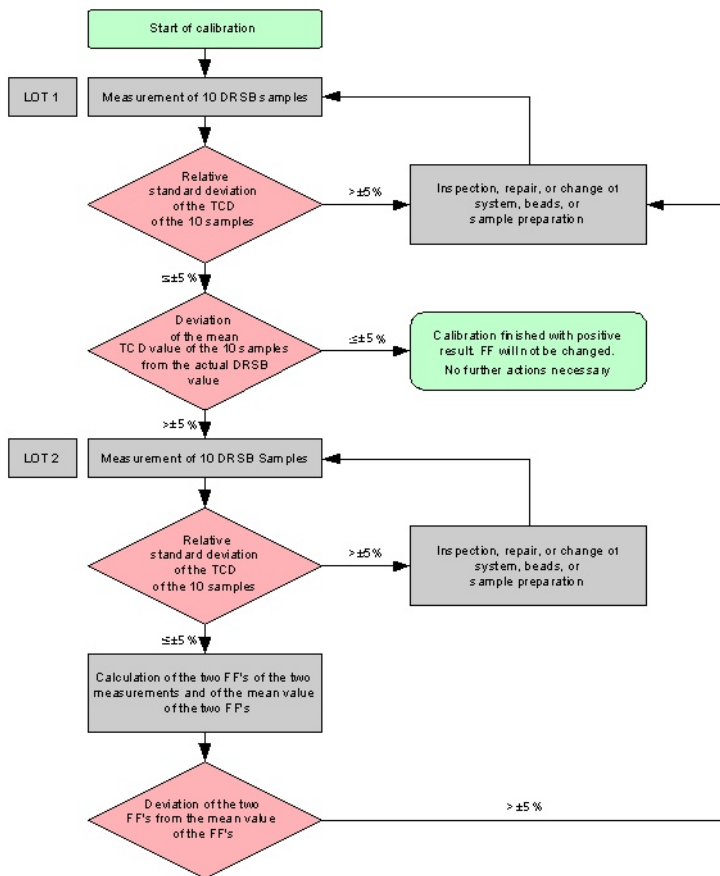


Fig. 2: Calibration Cedex HiRes Analyzer

6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

To make information consistent and easier to read, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ②, <i>etc.</i>	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, <i>etc.</i>	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

6.1.2 Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
④	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Updated to include lot-specific data for new lot.
- Chapter 4, Lot Specific Data: removed reference to print button.
- Editorial changes.

6.3 Trademarks

CEDEX is a trademark of Roche.

All other product names and trademarks are the property of their respective owners.

6.4 Regulatory Disclaimer

For use in quality control/manufacturing process only.

6.5 Online Technical Support

Please visit our Online Technical Support site for additional information about this product:

www.technical-support.roche.com

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For more documentation such as instructions for use and safety data sheets, please visit custombiotech.roche.com**

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